THE ESTABLISHMENT, BIOLOGICAL SUCCESS AND HOST IMPACT OF *Diorhabda elongata*, IMPORTED BIOLOGICAL CONTROL AGENTS OF INVASIVE *Tamarix* IN THE UNITED STATES

A Thesis

by

JEREMY L. HUDGEONS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2007

Major Subject: Entomology

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Approved by:

Co-Chairs of Committee,	Kevin M. Heinz
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ABSTRACT

The Establishment, Biological Success and Host Impact of *Diorhabda elongata*, Imported Biological Control Agents of Invasive *Tamarix* in the United States.

(August 2007)

Jeremy L. Hudgeons, B.S., Texas Tech University

Co-Chairs of Advisory Committee: Dr. Kevin M. Heinz
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Diorhabda elongata elongata leaf beetles were released at two field locations in the upper Colorado River watershed of Texas in 2003 and 2004 for the biological control of invasive *Tamarix*, exotic trees deteriorating riparian ecosystems of western North America. Establishment and biological success were monitored using trees on transects from the release points. *D. elongata elongata* released at the Lake Thomas site in August 2003 successfully overwintered and were recovered in the spring 2004; however, beetles were not present after June 2004. The April 2004 release at Beals Creek led to establishment and survival during 2005 and 2006. Mean abundance increased from less than five insects per tree per 2 minute count in August 2004 to more than 40 insects per tree per 2 minute count in August 2006. By then the population was dispersed throughout an area of approximately 12 hectares and beetles were present on 100% of the 47 trees surveyed, 57% of which were at least 90% defoliated.

To measure the impact of beetle defoliation on *Tamarix*, nonstructural carbohydrates (NCHOs) were measured in manipulative field cage experiments in Texas and natural experiments in Nevada. There was no significant difference in NCHOs

between trees with versus trees without beetle herbivory in the cage experiment, although spring foliage regrowth was reduced by 35% in trees defoliated the previous fall. In Nevada, root crown tissue was sampled in 2005 and 2006 from trees that had experienced 0-4 years of defoliation. In 2005, NCHO concentrations differed between tree stands and ranged from 9.0 ± 0.8% (Mean ± SE) in non-defoliated trees to 3.2 ± 0.4%, 2.1 ± 0.4% and 2.3 ± 0.4% in trees defoliated for 1, 2 and 3 successive years, respectively. NCHO concentrations in 2006 were similar, ranging from 13.6 ± 0.9% in non-defoliated trees to 7.6 ± 0.8%, 2.3 ± 0.4%, 1.5 ± 0.3% and 1.7 ± 0.4% in trees defoliated for 1, 2, 3 and 4 years, respectively. The establishment, biological success and host impact of *D. elongata* leaf beetles suggest there is potential for biological control of *Tamarix* in the United States.

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CHAPTER I

INTRODUCTION

Background

One of the most serious invasions of a noxious weed within the United States involves species of the exotic genus *Tamarix* L. (Stein and Flack 1996). *Tamarix* is one of four genera in Tamaricaceae (Tamaricales) and is represented by 54 species, none of which occur naturally in the Western Hemisphere (Baum 1967). The genus arose during the Cretaceous Period in the Central Asian deserts (Pakistan – Afghanistan – Iran – Turkmenistan – southern Kazakhstan – western China) where it adapted particularly well to saline soils of riparian habitats (Kovalev 1995). The native range of the genus extends from its northern extremes in China and Mongolia southward into India, circumvents the Mediterranean regions from the Middle East through southern Europe to Spain, and across northern Africa and along eastern Africa to southern Africa (Baum 1978).

Tamarix species are woody perennial trees, having a well developed trunk, or multi-stemmed shrubs (Baum 1978). The plants can grow between one and twelve meters tall depending on water availability, elevation and species (Everitt 1980). As riparian species, the plants are found in direct association with a stream channel or its immediate flood plain, yet they are very adaptable and tolerant of a wide range of environmental conditions. This ability to adapt to variable conditions is in part due to

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Tamarix being facultative phreatophytes, meaning they are able to obtain their water from both phreatic (groundwater or capillary fringe of the groundwater table) and vadose (unsaturated soil moisture) zones (Smith *et al*. 1998). *Tamarix* are also facultative halophytes which are capable of tolerating soluble salt concentrations ranging between 650 and 16,000 ppm and averaging approximately 6,000 ppm (Carman and Brotherson 1982, Brotherson and Winkel 1986). *Tamarix* can be found at elevations below sea level or at elevations above 2000 meters (Everitt 1980). The plants can propagate sexually (via seeds) and vegetatively (Everitt 1980). *Tamarix* can produce a prodigious number of seeds (up to 500,000 per tree per year) virtually throughout the growing season from May to October (Brotherson and Field 1987). The tiny seeds are equipped with a tuft of hair allowing for wind distribution, but may also be carried and deposited along river channels by water (Everitt 1980). In addition, new plants can develop from adventitious rooting of submerged or buried stems (Everitt 1980).

Tamarix Invasion

The oldest known references of *Tamarix* in North America are from a nursery catalogs published in New York in 1823 and in California in 1856 (Horton 1964). During the early years of its introduction, *Tamarix* plants were grown as ornamentals, planted to create wind breaks, provide shade, and stabilize eroding stream banks (Neill 1985). By the 1920s the plants had escaped cultivation and were becoming a serious ecological threat (Brotherson and Field 1987).

Between eight and twelve species of *Tamarix* have been introduced into North America since the early 1820s (Baum 1967, Crins 1989). All introduced species except

Tamarix aphylla (L.) Karsten are deciduous and commonly referred to as saltcedar or tamarisk. There is some controversy regarding the identity and corresponding native range of the majority of invasive *Tamarix* species (Gaskin and Schaal 2003). With the exception of *T. aphylla* and *Tamarix parviflora* De Candolle, most naturalized species are difficult to distinguish morphologically (Crins 1989). The invasion by *Tamarix* in the United States may represent a species complex (Gaskin and Schaal 2003), and this complex has invaded over 500,000 hectares of riparian habitat in the western United States (Robinson 1965). *Tamarix* continues to expand its range as far north as Montana and is believed to be capable of extending into the plains of Canada (Pearce and Smith 2003).

Many factors have been attributed to the North American invasion by *Tamarix*. Anthropogenic alterations of western riparian ecosystems including dam and reservoir construction, river diversions, flow regulations, native phreatophyte control programs and agricultural activity contributed to creating ideal conditions for *Tamarix* invasion of disturbed areas (DiTomaso 1998, DeLoach *et al*. 2000). The many innate biological characteristics of *Tamarix* mentioned previously, including its prolific seed production, vegetative reproduction capacity, tolerance of wide range of environmental conditions, interspecific competition through salt excretion and rapid recovery from fire have also contribute to its success as an invasive weed (Brotherson and Field 1987). Finally, the release from natural enemy regulation in North America is hypothesized to aid in the *Tamarix* invasion (DeLoach *et al*. 1989).

The impacts associated with the *Tamarix* invasion in North America include their damage to native plant communities, effects on wildlife, stream channel modification, deterioration to outdoor recreational areas and high water usage. The ability of *Tamarix* to function as facultative phreatophytes in an arid floodplain has resulted in a shift in species composition from native cottonwoods (*Populus* spp.) and willows (*Salix* spp.), obligate phreatophytes which historically dominated much of the vegetation along riparian ecosystems of the arid southwestern United States (Smith *et al*. 1998). Furthermore, *Tamarix* can utilize high saline groundwater and excrete excess salts through leaf glands, which then accumulate on the soil surface. Excessive deposits of salt can reduce the growth or inhibit germination of native non-halophytic species, thereby further restricting competition with other understory and overstory vegetation for space and water (Brotherson and Field 1987). As a result, the plants commonly form near monotypic stands where they grow and reach densities of 700-1000 plants per hectare in some regions (Ellis 1995, Hart *et al*. 2005).

Most wildlife species are adversely affected by the displacement of native vegetation by *Tamarix*. A few native or naturalized insects thrive on introduced *Tamarix* including the cicada (*Diceroprocta apache* Davis) and the honey bee (*Apis mellifera* L.) (Horton and Campbell 1974). The leafhopper (*Opsius stactogalus* Fieber) which was introduced into the US from Eurasia by unknown means can reach high population numbers during the growing season. In general, however, native plant communities support a greater diversity of insect life than does introduced *Tamarix* (Neill 1985). Insect diversity at the family and species level are greatly reduced in

Tamarix stands when compared to native willow (*Salix interior* Rowlee) and seep-willow (*Baccharis salicina* Torr. & Gray) stands (Knutson *et al*. 2003).

Measure of total bird density and species diversity are often lower in *Tamarix* stands when compared to native plant communities (Anderson *et al*. 1977, Kerpez and Smith 1987). Johnson (1987) found that native riparian areas along the Colorado River sustained a density of 154 birds per 40 hectares; whereas the *Tamarix* dominated areas sustained only four birds per 40 hectares. Some obligate riparian bird species can successfully utilize *Tamarix* stands (Ellis 1995). While the southwestern willow flycatcher (*Empidonax trailii extimus* Phillips) breeds in a diverse array of riparian habitats, it now nests extensively in *Tamarix* in some areas in Arizona (Sogge and Marshall 2000). In 1998, 77.6% of *E. t. extimus* nests in Arizona were in *Tamarix* trees (Paradzick *et al*. 1999). Because *E. t. extimus* is protected by federal law as an endangered species (U.S. Fish and Wildlife Service 1995), the effects of *Tamarix* management on southwestern willow flycatcher populations has raised some concerns in terms of conflicts of interest. DeLoach *et al*. (2000) have argued that control of *Tamarix* and the subsequent restoration of native plant communities are not expected to negatively impact native species (DeLoach *et al*. 2000); however, monitoring and protecting flycatcher populations in Arizona during the restoration process will be important.

Tamarix stands are used for vegetational cover by some mammal species including feral hog (*Sus scrofa* L.); however, with the exception of woodrats (*Neotoma* spp.) and the desert cottontail (*Sylvilagus audubonii* Baird), no other native mammal

species are known to feed on mature *Tamarix* plants (DiTomaso 1998). Thirty-four species of fish which are federally listed as either threatened or endangered by the U.S. Fish and Wildlife Service occur in areas infested by *Tamarix* (DeLoach *et al*. 2000). *Tamarix* degrades fish habitat by reducing water levels, modifying stream morphology (see below) and shading. Removal of *Tamarix* along one springbrook in Nevada increased the density of the endangered desert pupfish (*Cyprinodon pecosensis* Echelle & Echelle) (Kennedy *et al*. 2005).

Alteration in stream hydrology is often caused by the dense stands of *Tamarix* which form on the stream banks. The dense stands slow river flow which increases sedimentation and bank aggradation (Brotherson and Field 1987). As the river recedes, the plants establish themselves further into the channel, reducing normal stream flow and exacerbating the process. The result is a narrowing and deepening of the channel which can increase the incidence and severity of flooding (Blackburn *et al*. 1982). Furthermore, invasive *Tamarix* reduces recreational usage of parks and other outdoor areas for boating, camping, hiking, hunting, fishing, wildlife watching and photography (DeLoach 1991). This occurs not only because *Tamarix* causes declines in species diversity but also because the near impenetrable *Tamarix* stands block access to recreational waters.

A number of factors affect the volume of water transpired by *Tamarix* including time of season, weather conditions, plant density and size, salinity, soil type and depth to water table (Davenport *et al*. 1982, Devitt *et al*. 1997, White *et al*. 2003). Estimates of *Tamarix* evapotranspiration (ET) vary depending on the method of measurement, study

location and length of study (Hays 2003). *Tamarix* ET in July varied from 22 to 158 cubic meters per hectare per day depending on stand density; the latter estimate was roughly 2.4 times the ET rate of grass during the same period (Davenport *et al*. 1982). Hays (2003) estimated the May through October growing season water use by *Tamarix* at locations along the Canadian, Colorado and Pecos Rivers in Texas. Water use varied from 39 cubic meters per hectare per day to 210 cubic meters per hectare per day depending on depth to groundwater, soil texture, specific yield and stand density (Hays 2003). Conservative economic estimates of the impact of water losses to western United States municipalities, agriculture and hydropower generation total $280-450 per hectare of *Tamarix* (Zavaleta 2000).

Control of Invasive *Tamarix*

Tamarix is necessary to begin restoring riparian habitats to their pre-invasion conditions in North America. Various strategies have been employed to manage *Tamarix* including burning, mechanical removal and chemical control. Control of *Tamarix* by fire is ineffective because *Tamarix* sprouts readily from below ground vegetative structures (Anderson *et al*. 1977, Busch and Smith 1993). The ability of the *Tamarix* to reproduce vegetatively from pieces of stems and roots predisposes mechanical control through cutting and shredding to be a failure. Effective mechanical control requires removing the root crown from the soil, raking to remove stems and burning removed material (McDaniel and Taylor 2003). A second mechanical clearing is often needed to reach satisfactory control; the combined treatments are labor intensive and costly, exceeding $1000 per hectare (McDaniel and Taylor 2003). Chemical control

of *Tamarix* has historically been unsatisfactory (Stevens and Walker 1998, Duncan and McDaniel 1998). However, recent research indicates that the herbicide imazapyr (Arsenal™, BASF, Ludwigshafen am Rhein, Germany) applied alone (1.12 kg a.i./ha) or in combination with glyphosate (Rodeo™ or Roundup™, Monsanto, St. Louis, MO, USA) (0.56+0.56 kg a.i./ha of imazapyr and glyphosate) provides more than 84% mortality of *Tamarix* (Duncan and McDaniel 1998, Hart *et al*. 2005). Large scale herbicide treatments using imazapyr are now underway along the Pecos and Colorado River systems in Texas (Hart *et al*. 2005, McGinty *et al*. 2006). However, chemical control alone is not desirable for several reasons including (1) its ability to kill non-target plant species makes chemical treatment in highly mixed vegetation difficult, (2) its less than complete effectiveness allows potential re-infestation of a treated area and (3) the economic costs prohibit repetitive treatment applications.

Biological Control of *Tamarix*

To help combat the *Tamarix* invasion, the United States Department of Agriculture –Agricultural Research Service (USDA-ARS) initiated an importation biological control research program in the late 1960s by directing overseas surveys to identify potential control agents (DeLoach *et al*. 2003). Pre-release natural enemy evaluations were conducted at the USDA-ARS Arthropod Containment Facility at Temple, Texas starting in 1986 (DeLoach *et al*. 2000). Classical biological control of invasive plants is the importation and release of exotic insects, mites or pathogens for the purpose of reducing the vigor, reproductive capacity, or density of weeds. The premise behind classical biological control is that natural enemies limit population growth of

their target plants in native regions and the introduction of these enemies will therefore limit the population growth of the invasive species in introduced areas. Overseas surveys list more than 300 species of insects and other arthropods that feed on *Tamarix* in its native range; only five of these *Tamarix* feeding arthropods have been introduced into the US by unknown means, none of which are providing substantial natural control (DeLoach *et al*. 2003). The high ecological threat of *Tamarix*, the lack of taxonomically related plants in North America, the abundance of host-specific and damaging insects that attack *Tamarix* in its native range and the lack of natural enemies in its invasive range make the weed an ideal candidate for biological control.

Biological control of *Tamarix* is not expected to be rapid. Successful control at given sites will probably require the introduction of several agents and 5 to 10 years time; successful control may not be possible in all invaded areas (DeLoach 1996). Impacts to non-targets are also a concern in *Tamarix* biological control. Host range testing of a potential biological control agent is necessary to ensure important native and agricultural plants are not damaged (see below). And as mentioned previously, any control of *Tamarix* in Arizona will necessitate protecting *E. t. extimus* populations.

Biological Control Agents

The leaf beetle *Diorhabda elongata* (Brullé) *sensu lato* (Coleoptera: Chrysomelidae) is one of the first biological control agents investigated in the USDA-ARS biological control program. Populations of *D. elongata* are found throughout most of the native range of *Tamarix*. Populations of *D. elongata* collected to date for study within the biological control program originate from Turpan and Fukang, Xinjiang

Province, China; Chilik, Kazakhstan; Sfax, Tunisia; Sfakaki, Crete and Kalandra, Greece; and Karshi (Qarshi), Uzbekistan, (DeLoach *et al*. 2003, Milbrath and DeLoach 2006b). The broad geographic distribution of *D. elongata* in the Old World is expected to translate to populations which can adapt to geographic conditions at release areas in North America.

The biology of *Diorhabda elongata* (Brullé) *deserticola* Chen has been described in detail (Lewis *et al*. 2003b). The adult and all three larval instars feed on the foliage of *Tamarix*. When fully grown, the third instar larvae crawl or drop to the ground and pupate in the underlying leaf litter. The adult stage overwinters in the leaf litter and becomes active coincident with *Tamarix* budbreak in the spring. Female lifetime fertility average 194 eggs; females glue the eggs to the host foliage singly or, more typically, in masses ranging from two to twenty (Lewis *et al*. 2003b). In its native range, *D. elongata deserticola* herbivory can cause heavy to complete defoliation of *Tamarix* (DeLaoch *et al*. 2003).

Host range studies confirmed that *D. elongata deserticola* is sufficiently host-specific to the genus *Tamarix*. Of native North American plants, those nearest related to *Tamarix* are six species of *Frankenia* (family Frankeniaceae: order Tamaricales) which occur mostly in the desert areas of the southwestern United States and northern Mexico (Whalen 1980, Whalen 1987); besides the native *Frankenia* and the introduced *Tamarix*, no other plant species within the order Tamaricales are found in North America. Laboratory and field trials demonstrated that *D. elongata deserticola* survival from larvae to adult is significantly reduced on *Frankenia* and that these plants are poor hosts

for the agent (DeLoach *et al*. 2003, Lewis *et al*. 2003a). Other native and agricultural plants included in the tests did not support larval development (DeLoach *et al*. 2003).

Due to its broad geographic range in the Old World, presumed adaptability in the United States, ability to defoliate trees in natural conditions and high host specificity, *Diorhabda elongata deserticola* was recommended for the biological control of *Tamarix* in 1994 (DeLoach 1994, DeLoach *et al*. 2003). The USDA-Animal and Plant Health Inspection Service (APHIS) issued permits for release of this agent into field cages in 1999 (DeLoach *et al*. 2003). Subsequently, APHIS issued permits for limited open field releases at ten research sites in six western states (California, Nevada, Utah, Wyoming, Colorado and Texas), and the first releases were made at eight of these sites in May and June 2001 (DeLoach *et al*. 2003).

Evaluation of Biological Control Agent

Harris (1991) divides the progress of a biological control program into four steps: establishment, biological success, host impact and control success. Harris (1991) defines establishment as the recovery of the biological control agent for at least two years following its release into the open field. Biological success is a measure of resource use by the agent in relation to the resource available. An agent that remains rare and consumes little of its host resources in relation to available resources contributes little to control and is considered a failure. Host impact is a measure of the decrease of reproduction or biomass of the weed at sites where the agent is established. Control success relates to the objectives of the project and can reflect environmental, economic and human values in addition to the impact of control on weed density.

The establishment of natural enemies following their release is the first step to a successful classical biological control program. *D. elongata deserticola* from Fukang, China (latitude 44.16°N, longitude 87.98°E) and Chilik, Kazakhstan (latitude 43.33°N, longitude 78.25°E) was the first biological control agent investigated at the APHIS approved sites. The beetles successfully overwintered at sites north of the 38th parallel, but the beetles failed to overwinter at the two most southern sites near Hunter-Liggett Military Base, California (latitude 35.95°N, longitude 121.30°W) and Seymour, Texas (latitude 33.58°N, longitude 99.26°W). Investigators determined that the most probable cause of the failure to overwinter was the shorter summer daylength at the southern sites (Lewis *et al.* 2003b, Bean *et al*. 2007). The maximum summer daylengths at Fukang, China and Chilik, Kazakhstan are at least 15h 20min, whereas the maximum summer daylengths at the California and Texas sites are less than 14h 40min which was determined to be a critical photoperiod for these beetle populations (Bean *et al*. 2007). The shorter daylength induced the beetles to enter diapause in July at the southern sites; as a consequence, the beetles exhausted their fat reserves during fall and winter and starved before *Tamarix* foliage appeared in March (Lewis *et al*. 2003b).

More southern populations of *Diorhabda elongata sensu lato* have shorter critical photoperiods for diapause induction (Bean *et al*. 2004), and thus are more likely to establish at release sites in more southern latitudes in North America. *Diorhabda elongata elongata* (Brullé) from Crete, Greece (latitude 35°15'N, longitude 24.6°E) has since been approved for release in the United States. Under natural conditions, the critical photoperiod for diapause induction in *D. elongata elongata* is approximately 12

hours (Bean *et al*. 2004). The first objective of our study is to evaluate the establishment and biological success of *D. elongata elongata* at release sites within the upper Colorado River watershed in Texas.

Once established and biologically successful, the biological control agent must exert enough herbivore pressure on the target weed to decrease its biomass or reproductive capacity. Leaf-chewing chrysomelid beetles have been used with great successes in biological control programs (Crawley 1989). Within ten years of its introduction in 1946, *Chrysolina quadrigemina* (Suffrian), and to a lesser degree, *Chrysolina hyperici* (Förster), reduced klamath weed, (*Hypericum perforatum* L.) abundance to less than one percent of its former occurrence in California with a concurrent increase in native grasses (Huffaker and Kennett 1959). Herbivory by *Calligrapha pantherina* Stål has provided substantial to complete control of spinyhead sida (*Sida acuta* Burman f.) in coastal regions of Australia (Flanagan *et al*. 2000).

Some workers have proposed that leaf-chewing insects have been successful biological control agents because the removal of photosynthetic tissue by defoliation reduces the ability of plants to maintain growth and vigor. The biological control of perennial woody plants such as *Tamarix* is thought to be more difficult. The large food reserves of woody plants often enable them to re-foliate after herbivory by defoliating insects. However, several examples of successful biological control of woody plants have been reported. The leaf beetle *Metrogaleruca obscura* Degeer and the eurytomid gall wasp *Eurytoma attiva* Burks have successfully controlled the woody shrub, black sage (*Cordia macrostachya* (Jacquin)) in Mauritius (Fowler *et al*. 2000) and Maylasia

(Simmonds 1980). The leguminous, woody tree *Sesbania punicea* (Cav.) Benth has been successfully controlled in South Africa by the combined effects of three introduced weevil species: *Trichapion lativentre* (Beguin-Billecocq) which primarily destroys flower buds, *Rhyssomatus marginatus* Fahraeus which destroys the developing seeds, and *Neodiplogrammus quadrivittatus* (Oliver) whose larvae bore into the trunk and stems (Hoffman and Moran 1998). Though three insect species combined to control *Sesbania punicea*, Hoffman and Moran (1998) emphasize that it is the impact the agents have on the target weed rather than the quantity of insect species that ultimately holds the key to reductions in host plant densities.

Tamarix biological control workers have observed *D. elongata* to completely defoliate *Tamarix* trees. However, the consequences of *D. elongata* herbivory on *Tamarix* growth and vigor are largely unknown. To recover from defoliation, plants need adequate carbohydrate reserves to regenerate new leaf tissue (Loescher *et al*. 1990, Chapin *et al*. 1990). Furthermore, stored carbohydrates serve important roles in metabolism, growth, development of cold hardiness, defense and the survival of woody plants (Kozlowski 1992). Maintenance respiration in living cells when photosynthesis is low or has stopped due to defoliation or deciduousness is dependent on adequate carbohydrate reserves, as is new spring leaf growth in all deciduous species (Loescher *et al*. 1990, Kozlowski 1992). Thus a reduction in carbohydrate reserves in *Tamarix* by *D. elongata* defoliation could lower plant growth and vigor.

The removal of photosynthetic tissue by defoliation lowers carbohydrate storage reserves in plants. Reduction in carbohydrate reserves following artificial defoliation of

eight salt-desert shrub species may be the result of continued respiration, reduction in photosynthesis and the use of reserves in producing regrowth (Trlica and Cook 1971). Reserve carbohydrates were reduced while supporting new leaf growth following artificial defoliation of honey mesquite (*Prosopis glandulosa* Torr.) (Cralle and Bovey 1996). Contrary to these results, the transient effects on carbohydrate reserves and the rapid recovery of growth revealed the tolerance of healthy stands of hybrid poplar (*Populus* X *canadensis* cv Eugeneii) to outbreaks of the defoliating gypsy moth (*Lymantria dispar* L.) (Kosola *et al*. 2001). Whether *Tamarix* will show a similar pattern in carbohydrate reserves and regrowth following defoliation by *D. elongata* needs to be investigated.

Diorhabda elongata deserticola has established at northern release sites in the United States. Evaluating the establishment, dispersal and biological success of *Diorhabda elongata elongata* at release sites in Texas will aid in understanding the adaptability of the beetle to habitats invaded by *Tamarix* in the southern regions of the United States. Further, monitoring the effects of *D. elongata sensu lato* defoliation on *Tamarix* carbohydrate reserves and regrowth will indicate the degree of host impact the agent has on the target weed. These studies will give insight to the potential for control success of *Tamarix* in the United States.

Research Objectives

This study focused on evaluating the efficacy of *Diorhabda elongata* (Brullé) *sensu lato* leaf beetles as biological control agents against *Tamarix* in the United States. The specific objectives were to (1) evaluate establishment and biological success from releases of *Diorhabda elongata elongata* (Brullé) which were made in the upper Colorado River watershed of Texas during 2003 and 2004 and (2) measure host impact by quantify the effects of *D. elongata elongata* and *D. elongata deserticola* defoliation on *Tamarix* carbohydrate reserves and regrowth from trees in field cage and natural experiments conducted between 2004 and 2006 in Texas and Nevada.

CHAPTER II

ESTABLISHMENT AND BIOLOGICAL SUCCESS OF *Diorhabda elongata elongata* ON INVASIVE *Tamarix* IN TEXAS

Introduction

One of the most serious invasions of a noxious weed within the United States involves species of the exotic genus *Tamarix* L. (Tamaricales: Tamaricaceae) (Robinson 1965, Stein and Flack 1996, DeLoach *et al*. 2000). *Tamarix* species are woody perennial trees or multi-stemmed shrubs native to arid riparian habitats of Eurasia and Africa (Baum 1978). *Tamarix* are facultative phreatophytic (capable of obtaining water from both the phreatic and vadose zones) (Smith *et al*. 1998) and facultative halophytic species (capable of tolerating soluble salt concentrations ranging between 650 and 16,000 ppm) (Carman and Brotherson 1982, Brotherson and Winkel 1986). The plants are able to produce a prodigious number (up to 500,000 per tree per year) of tiny seeds throughout the growing season which are equipped with a pappus allowing for wind distribution, but may also be carried and deposited along river channels by water (Brotherson and Field 1987, Everitt 1980).

The oldest known references of *Tamarix* in North America are from nursery catalogs published in New York in 1823 and in California in 1856 (Horton 1964). During the early years of its introduction, *Tamarix* trees were grown as ornamentals, planted to create windbreaks, provide shade, and stabilize eroding stream banks (Neill 1985). By the 1920s *Tamarix* species had escaped cultivation and were becoming a serious threat to arid riparian ecosystems in North America (Brotherson and Field 1987).

Between 8 and 12 species of *Tamarix* have been introduced into North America since the early 1820s (Baum 1967, Crins 1989). All introduced species except *Tamarix aphylla* (L.) Karsten (athel trees) are deciduous and commonly referred to as saltcedar or tamarisk. The invasion by *Tamarix* in the United States represents a complex of 4 to 6 species and their hybrids (Gaskin and Schaal 2003), and this complex has invaded over 500,000 hectares of riparian habitat in the western United States (Robinson 1965). *Tamarix* continues to expand its range south into northern Mexico (González y Aldape 1991) and as far north as Montana and may be capable of extending into the plains of Canada (Pearce and Smith 2003).

The negative effects of the *Tamarix* invasion far exceed the desirable qualities associated with their original introduction and cultivation. The plants commonly form monotypic stands where they grow and reach densities of 700-1000 plants per hectare in some regions (Ellis 1995, Hart *et al*. 2005). In most cases, faunal diversity is reduced in *Tamarix* stands when compared to native vegetation (Anderson *et al*. 1977, Kerpez and Smith 1987**,** Knutson *et al*. 2003). Furthermore, the dense stands along river banks and floodplains can result in increased sedimentation and bank aggradation (Brotherson and Field, 1987), a narrowing of the channel with increased incidence of flooding (Blackburn *et al*., 1982) and a lowering of water tables (Smith *et al*. 1998).

To help combat the *Tamarix* invasion, the United States Department of Agriculture – Agricultural Research Service (USDA-ARS) initiated an importation biological control research program in the late 1960s by directing overseas surveys to identify potential control agents (DeLoach *et al*. 2003). Pre-release natural enemy

evaluations were conducted at the USDA-ARS Arthropod Containment Facility at Temple, Texas starting in 1986 (DeLoach *et al*. 2000). One result of this program was the pre-release screening and approval for release of *Diorhabda elongata* (Brullé) *sensu lato* (Coleoptera: Chrysomelidae) (DeLoach *et al*. 2003). The adult and all three larval instars feed on the foliage of *Tamarix*. When fully grown, the third instar larvae crawl or drop to the ground and pupate in the underlying leaf litter. Two and sometimes three generations can be produced in North America, prior to entrance of adults into reproductive diapause in response to shortened daylength (Bean *et al*., 2007). The adult stage overwinters in the leaf litter and becomes active around spring budbreak. Females oviposit 128 - 280 eggs in masses (3-8 eggs per mass) on the host foliage (Lewis *et al*. 2003, Milbrath *et al.* 2007). In its native range, *D. elongata* herbivory can cause heavy to complete defoliation of *Tamarix* (DeLoach *et al*. 2003).

The establishment of a natural enemy following its release is the first step for a successful classical biological control agent. *Diorhabda elongata deserticola* Chen from near Fukang, China (ca. 50 km NNE of Urumqi, latitude 44.16°N, longitude 87.98°E, elevation 567 m) and near Chilik, Kazakhstan (120 km ENE Almaty, latitude 43.33°N, longitude 78.25°E, elevation 662 m) successfully overwintered in the U.S. at sites north of the 38th parallel, but the beetles failed to overwinter at the two most southern sites: near Hunter-Liggett Military Base, California (latitude 35.95°N, longitude 121.30°W) and at Seymour, Texas (latitude 33.58°N, longitude 99.26°W). Investigators determined that the most probable cause of the failure to overwinter was the shorter summer daylength at the southern sites (Lewis *et al.* 2003, Bean *et al*. 2007). The shorter

summer daylength induced the beetles to enter diapause in early summer at the southern sites; as a consequence, the beetles exhausted their metabolic reserves during fall and winter and starved before *Tamarix* foliage appeared in March (Lewis *et al*. 2003).

Subsequently, *Diorhabda elongata elongata* (Brullé) from Crete, Greece (latitude 35°15'N, longitude 24.6°E) was imported into the United States. This southern adapted species has shorter critical photoperiod requirements for diapause induction (Bean *et al*. 2004), and thus is more likely to establish at release sites in more southern latitudes in North America. The objective of this study is to measure the establishment and biological success of the leaf beetle *Diorhabda elongata elongata* at two sites within the upper Colorado River watershed in northwestern Texas. Establishment was defined as the recovery of the biological control agent for at least 2 years following its release into the open field and biological success is the measure of resource use by the agent in relation to the resource available (Harris 1991). Biological success was determined by assessing the frequencies of *Tamarix* trees occupied and defoliated by *D. elongata elongata*. In addition to establishment and biological success, measures of dispersal distances and rates were calculated at the release sites.

Materials and Methods

Species Released

The insects released in this study originated from approximately 200 adults collected on *Tamarix* species 3km west of Sfakaki, Crete, Greece (latitude 35.38°N, longitude 24.6°E, elevation 7m) in April 2002. Insects were identified as *Diorhabda elongata elongata* (Brullé) by I.K. Lopatin (Professor, Byelorussian University, Minsk,

Belarus). The beetles were exported from Crete to the USDA-ARS Exotic and Invasive Weed Research Unit quarantine facility at Albany, California. Beetles (eggs and adults) were subsequently sent to the USDA-ARS Arthropod Containment Facility at Temple, Texas and maintained on cultivated *Tamarix* in lab and field cages in Temple since June 2002. Field cages measured 3.5 m by 2.8 m by 2.6 m high and were covered by 32 X 32 woven high-density polyethylene fabric insect screening (PAK Unlimited Inc., Cornelia, GA). In July 2003, APHIS approved limited open field releases of *D. elongata elongata* in Texas. Subsequently, field cages were erected over individual *Tamarix* trees in Borden and Howard counties in July of 2003 in order to rear and amass *D. elongata elongata* insects for open field releases within the upper Colorado River watershed.

Release Sites

D. elongata elongata were released at two sites termed "Lake Thomas" and "Beals Creek", both located within the upper Colorado River watershed (Fig. 1). The Lake Thomas site is on the western end of Lake J. B. Thomas in Borden County, Texas. The current drought affecting the area has reduced the lake's storage to below 20% of its 24,600 ha-m (200,000 acre-ft) capacity and much of the former lake bottom has been invaded by *Tamarix* trees. The first open field release of *D. elongata elongata* in Texas was made at this site (latitude 32.60° N, longitude 101.21° W, elevation 677 m) on 21 August 2003 (Table 1). Soil samples collected from the surface to 15 cm (6 in) deep were analyzed by the Soil, Water and Forage Testing Laboratory at Texas A&M University (College Station, Texas) and found to be 40% clay, 50% silt and 10% sand. The vegetation at this site consists primarily of *Tamarix* species or hybrids (3-6 m in

height), willow baccharis (*Baccharis salicina* Torr. and Gray), *Chloracantha spinosa* (Benth.) Nesom and *Lippia nodiflora* (L.) Michx. with lesser amounts of Bermuda grass (*Cynodon dactylon* (L.) Pers.) and western ragweed (*Ambrosia psilostachya* DC.). On the fringe of the site and occasionally in the site, there were honey mesquite (*Prosopis glandulosa* var. *glandulosa* Torr.) and willow trees (*Salix nigra* Marsh.).

The second release location, Beals Creek, is 9km ESE of Big Spring (latitude 32.25° N, longitude 101.38° W, elevation 714 m) in Howard County, Texas. The site is on private land and within the flood plain of Beals Creek, a tributary of the Colorado River. Soil samples were also collected and analyzed at this site as previously described and were found to be 63% sand, 31% silt, 6% clay. The dominant vegetation at Beals Creek is *Tamarix* species or hybrids (3-6 m in height), *P. glandulosa*, alkali sacaton (*Sporobolus airoides* Torr.), and saltgrass (*Distichlis spicata* var. *stricta* (L.) Greene) with lesser amounts of *B. salicina* and iodinebush (*Allenrolfea occidentalis* (Wats.) Kuntze). There is also some *C. dactylon* in patches and an occasional thicket of Berlandier's wolfberry (*Lycium berlandieri* Dunal).

All releases were made by the USDA-ARS and Texas A&M University in cooperation with the Colorado River Municipal Water District. Dates and number of insects released at each site are summarized in Table 1. Voucher specimens of *D. elongata elongata* were deposited to the Texas A&M University Insect Collection, College Station, Texas (under lot no. 663).

Insect Sampling

Transects were established along sixteen directions radiating out 200 m from the release point at each site. At 20 m intervals along each transect, the numbers of *D. elongata elongata* egg masses, larvae and adults were censused from a single *Tamarix* tree during a two-minute visual inspection of tree branches accessible from the ground by the census taker. Percent tree defoliation was also estimated by visual observation. If a tree did not occur at the 20 m mark along the transect, the nearest tree within 10 m in any direction was inspected. In cases where *Tamarix* trees were absent along a transect, no data were collected. Census data were entered into a GeoExplorer3 datalogger (Trimble, Sunnyvale, CA) and subsequently imported into a geographic information system (ESRI ArcView GIS 9.0, Redlands, CA) for observational analysis. Following transect establishment, surveys were generally conducted monthly between May and September during 2004 and 2005. Exceptions occurred when sites were inaccessible due to flooding or when logistical constraints prevented sites from being visited. The Beals Creek site was sampled on two additional dates in 2006.

For each census date, we report the total abundance of all *D. elongata elongata* life stages present (egg masses + larvae + adults) per all trees surveyed per 2 minute count. To determine whether insect abundance differed between the same months of successive years, the mean number of insects per tree among those months sampled more than once were examined using a Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA) followed by a nonparametric multiple comparison test (SPSS 11.5, Chicago, Illinois)**.**

The distance (D) from the release point for each observed *D. elongata elongata* insect stage was calculated by the Pythagorean theorem: $D = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$. In this equation, ($x_1 = 0$, $y_1 = 0$) is the position of the release tree and (x_2, y_2) is the position of the tree where the insect was observed. The mean dispersal distance for the population was calculated by summing the dispersal distances of all observed insects and dividing by the sum total of observed insects in each census. The maximum distance from the release point for an individual insect was noted in each census. The annual rate of dispersal for the population was calculated as the difference in mean dispersal distances per consecutive August and May months and is reported as meters per year. The relative frequency of transect trees occupied by *D. elongata elongata* at each sampling date was the number of trees with at least one insect stage divided by the number of trees surveyed. The relative frequency of defoliated transect trees was the number of trees more than 90% defoliated divided by the number of trees surveyed. In August of each year, the area occupied by defoliated trees within the *Tamarix* stand was mapped and calculated by circumnavigating this area with the GeoExplorer3 datalogger. Also, the number of trees within this area which were more than 90% defoliated was estimated.

Results

Lake Thomas

Before transects were established at Lake Thomas, preliminary surveys were conducted on the release tree and 19 surrounding trees in April and May 2004 to measure overwintering survival of beetles released the previous August. In April 2004,

D. elongata elongata egg masses, larvae and adults were found on 12 of the 20 surveyed trees (Table 2). Though census numbers were lower in May 2004, all stages of *D. elongata elongata* were found. Transects were established and 65 trees, including the release tree, were surveyed again in June 2004. Two adults were found on the release tree and two larvae and two adults were found on one tree 20 m from the release tree. Despite two additional releases of approximately 1000 adults at the original release tree in July (Table 1), five subsequent surveys through July, 2005 at Lake Thomas failed to recover any *D. elongata elongata* (Table 2).

The positions of the surveyed trees were not accurately recorded at the time of the initial surveys at Lake Thomas, thus mean dispersal distances could not be calculated for the *D. elongata elongata* insects found in April and May 2004. All 20 trees were within approximately 30 m of the site release tree, and thus 30 m is the estimated maximum dispersal distance at Lake Thomas.

Beals Creek

D. elongata elongata were released at Beals Creek in April and July 2004 (Table 1) at three locations. The locations of these releases were within an estimated 50 m of each other and on the western front of the *Tamarix* stand. By mid-July 2004, *D. elongata elongata* larvae and adults had defoliated two abutting *Tamarix* trees within 20 m of a field cage that was being used to rear beetles. Due to the concentration of *D. elongata elongata* at these two defoliated trees, this site was selected as the release point and origin of the transects. The location of the two defoliated trees on the western edge

of the *Tamarix* stand resulted in a less than perfect center release scenario with only eastern transects available for survey.

Forty-seven trees that fell on the transects were surveyed during each census. *D. elongata elongata* were recovered during every census over the next two years. Mean abundance of *D. elongata elongata* per tree per 2 min count at Beals Creek is summarized in Fig. 2. Following each overwintering period, *D. elongata elongata* abundance was low during the May censuses, but numbers increased by late summer of each year. The mean abundance in May 2006 was significantly higher than the mean abundance in May 2005 ($\chi^2 = 5.74$; df = 1; $P = 0.02$). Similarly, the mean abundance in August 2006 was significantly higher than mean abundance in August 2005 which was significantly higher than mean abundance in August 2004 ($\chi^2 = 61.10$; df = 2; $P < 0.001$).

The mean and maximum dispersal distances of the *D. elongata elongata* population at the Beals Creek release site are summarized in Table 3. The positions of the trees surveyed are illustrated in Fig. 3. The mean dispersal distance of the population generally increased during the census period. There was a slight retraction of the mean dispersal distance from October 2004 to June 2005 due in part to the low number of *D. elongata elongata* insects observed during the spring 2005 surveys. By August 2005, the mean dispersal distance of the *D. elongata elongata* population was approximately 55 ± 1.3 m from the release point, and insects were observed on the furthest transect tree surveyed, or 221 m from the release point (Fig. 3). By August 2006, the mean dispersal distance of the population was 128 ± 1.5 m from the release point. Seven random trees

were chosen to supplement the final survey. *D. elongata elongata* insects were observed on six of the seven trees, the furthest tree being located 375 m from the release point. Between August 2004 and August 2005, the *D. elongata elongata* population dispersed at a rate of 28 meters per year. Between May 2005 and May 2006 the rate of dispersal was 49 meters per year. Between August 2005 and August 2006 the rate of dispersal for the population had increased to 73 meters per year.

The majority of tree defoliation occurred in late summer and early fall when insect abundance was reaching its peak (Table 3). In August 2005, one year following the release, *D. elongata elongata* insects were present on 62% of the trees surveyed, and 21% of the surveyed trees were at least 90% defoliated (Table 3). Considering the entire *Tamarix* stand, an estimated 100 trees in a 0.4 hectare zone were at least 90% defoliated at this time. Though the defoliated trees generated new foliage in the spring of 2006, *D. elongata elongata* returned to defoliate the same trees later in the season. By August 2006, *D. elongata elongata* insects were observed on all 47 trees surveyed, 57% of which (27 trees) were at least 90% defoliated. The beetle population occupied at least 12 ha of the tree stand, and an estimated 400 trees covering 2 hectares nearest the release point were at least 90% defoliated. Figure 3 illustrates the defoliated area mapped during each August survey from 2004 to 2006.

Discussion

Harris (1991) divides the progress of a biological control agent into four steps: establishment, biological success, host impact and control success. Establishment is defined as the recovery of the biological control agent for at least 2 years following its

release into the open field (Harris 1991). Biological success is a measure of resource use by the agent in relation to the resource available. An agent that remains rare and consumes little of its host resources in relation to available resources contributes little to control and is considered a failure. Host impact is a measure of the decrease of reproduction or biomass of the weed at sites where the agent is established. Control success relates to the objectives of the project and can reflect environmental, economic and human values in addition to the impact of control on weed density.

Using the definition of establishment as the recovery of an agent for at least 2 years following its open field release (Harris 1991), *Diorhabda elongata elongata* established at one of two release sites in the upper Colorado River watershed. Though *D. elongata elongata* survived a winter at Lake Thomas, as can be attested by the presence of all life stages on trees the following April 2004, the population quickly declined as the season progressed, and no life stages were recovered past June 2004. Following their release in April and July 2004, a population of *D. elongata elongata* established at Beals Creek as adults survived two winters and were recovered during every survey through August 2006.

Aggregation behavior of the beetles may have played a part in the successful establishment at Beals Creek. Following three small releases totaling approximately 200 adults in the spring and early summer of 2004, two abutting trees were completely defoliated by *D. elongata elongata* in July. Though the defoliated trees were 50 m from where beetles were earlier released, they were within 20 m of the nursery cage used to rear beetles at the site. We surmise that adults in the open field were attracted to

chemical cues emitted from the caged insects and congregated and laid large numbers of eggs in the two *Tamarix* trees closest to the nursery cage. The absence of a nursery cage with beetles at Lake Thomas, where beetles failed to establish, supports this hypothesis. The tendency of conspecifics to aggregate following dispersal was noted in introductions of the chrysomelid beetle *Galerucella calmariensis* for the control of purple loosestrife (*Lythrum salicaria*) (Grevstad and Herzig, 1997). The authors suggested the possibility that the beetles may aggregate to ameliorate Allee effects such as predator dilution and increased availability of mates. Herzig and Root (1996) found that males of the chrysomelid beetle *Trirhabda virgata* colonized occupied patches of its goldenrod host (*Solidago altissima*) more frequently than unoccupied patches, suggesting that males aggregate to find mates.

The possibility of aggregation behavior in *D. elongata elongata* is further supported by the recent demonstration of an aggregation pheromone isolated from *D. elongata deserticola*. Cossé *et al*. (2005) analyzed volatiles collected from feeding male and female *D. elongata deserticola* adults and found two components produced almost exclusively by males. Subsequent field tests in their study demonstrated that males and females were attracted in roughly equal numbers to synthetic blends of the components indicating an aggregation pheromone produced by feeding males. Development of a synthetic *Diorhabda* aggregation pheromone and delivery system may improve the retention of adult populations of *D. elongata elongata* and the establishment rates at future release sites.

Though our survey methods were not intended to accurately estimate the number of beetles at the site, census data at Beals Creek is indicative of high mortality between fall and spring. However, the *D. elongata elongata* population experienced significant growth during the summer months, and the population grew each successive year. Beetle abundance in August 2006 was almost three and ten times greater than abundances in August 2005 and 2004, respectively. Furthermore, census data suggest that the *D. elongata elongata* population is dispersing at an increasing rate. From August 2004 to August 2005 and from August 2005 to August 2006, the population dispersed at average rates of 28 and 73 meters per year, respectively. However, this may be a conservative estimate of the population dispersal as it is based solely on census data from the 47 transect trees. By August 2006, *D. elongata elongata* could be found in trees along the periphery of the 12 ha *Tamarix* stand and in trees along Beals Creek, all of which were beyond the surveyed transect trees. These results suggest the beetles occupied at least 12 ha just 2 years following their release. The defoliated area had expanded to more than 2 ha and included an estimated 400 trees. Results from an aerial image taken on 19 September, 2006, reveal that *Tamarix* trees scattered about 7 ha around the release point were completed defoliated. Also, on 3 November 2006, adult and larvae *D. elongata elongata* were found on trees upstream on Beals Creek, the furthest insects being 1.7 km WNW of the release point. The relative frequencies of *Tamarix* trees occupied and defoliated by *D. elongata elongata* and the dispersal of the population give an indication as to the biological success of the beetle population at Beals Creek.

Increasing rates of expansion and major defoliation events have occurred at northern sites where *D. elongata deserticola* has been released. One year following a release near Lovelock, Nevada in 2001, *D. elongata deserticola* defoliated *Tamarix* trees covering 2 ha; the area of defoliation expanded to an estimated 120 ha of trees by the end of the 2003 growing season (Tom Dudley, Marine Science Institute, UC Santa Barbara, personal communication). Remote sensing assessment at the Lovelock release site estimated 1800 ha and 8100 ha of defoliated *Tamarix* by June 2004 and September 2005, respectively (Geraci 2006). Similarly, one year following a release near Moab, Utah in 2004, *D. elongata deserticola* defoliated an estimated 0.8 ha of *Tamarix*; the defoliated area had expanded to an estimated 44 ha by August 2006 (Swedhin *et al*. 2006).

This study is the first to document the successful establishment and biological success of *D. elongata elongata* in the United States. *D. elongata elongata* adults from the Beals Creek population were captured and re-released at 15 sites in 10 west Texas counties during the summer of 2006 as part of a Texas Cooperative Extension *Tamarix* integrative pest management program (Knutson *et al*. 2006). Results from these releases will further elucidate the potential for *D. elongata elongata* establishment in Texas. If defoliation by *D. elongata elongata* results in significant host impact in terms of reducing weed biomass or abundance at Beals Creek, there is promise for biological control success of *Tamarix* by *D. elongata elongata* at this site.

CHAPTER III

DEFOLIATION BY INTRODUCED *Diorhabda elongata* LEAF BEETLES REDUCES CARBOHYDRATE RESERVES AND REGROWTH OF *Tamarix*

Introduction

One of the most serious invasions of a noxious weed within the United States involves species of the exotic genus *Tamarix* L. (Tamaricales: Tamaricaceae) (Stein and Flack 1996, DeLoach *et al*. 2000). *Tamarix* species are woody perennial trees or multi-stemmed shrubs native to arid riparian habitats of Eurasia and Africa (Baum 1978). Between 8 and 12 species of *Tamarix* have been introduced into North America since the early 1820s (Baum 1967, Crins 1989). During the early years of its introduction, *Tamarix* trees were grown for a multiple purposes including: as ornamentals, for windbreaks, to provide shade, and to stabilize eroding stream banks (Neill 1985). By the 1920s *Tamarix* species had escaped cultivation and were becoming serious threats to arid riparian ecosystems in North America (Brotherson and Field 1987). All introduced species except *Tamarix aphylla* (L.) Karsten are deciduous and commonly referred to as saltcedar or tamarisk. The invasive taxa in the United States are *T. ramosissima* Ledeb., *T. chinensis* Lour., *T. parviflora* DC, *T. gallica* L. and hybrids of these (Gaskin and Schaal 2002). These species have invaded over 500,000 hectares of riparian habitat in the western United States (Robinson 1965). They continue to expand their range into northern Mexico (González y Aldape 1991) and as far north as Montana and may be capable of extending into the plains of Canada (Pearce and Smith 2003, Morisette *et al*. 2006). The *Tamarix* invasion is often reported to have negative ecological effects such

as displacement of native vegetation (Brotherson and Field 1987), reduction in faunal diversity (Anderson *et al*. 1977, Kerpez and Smith 1987**,** DeLoach *et al.* 2000**,** Knutson *et al*. 2003, Shafroth *et al*. 2005), increased sedimentation and bank aggradation (Brotherson and Field 1987), increased channelization (Blackburn *et al*. 1982) and a lowering of water tables resulting from high evapotranspiration rates (Smith *et al*. 1998 Nagler *et al*. 2005).

Biological Control Program

To help combat the *Tamarix* invasion, the United States Department of Agriculture –Agricultural Research Service (USDA-ARS) initiated an importation biological control research program in the late 1960s by directing overseas surveys to identify potential control agents (DeLoach *et al*. 2003). Pre-release natural enemy evaluations were conducted at the USDA-ARS Arthropod Containment Facility at Temple, Texas starting in 1986 (DeLoach *et al*. 2000). One result of this program was the pre-release screening and approval for release of *Diorhabda elongata* (Brullé) *sensu lato* leaf beetles (Coleoptera: Chrysomelidae) (DeLoach *et al*. 2003). The adult and all three larval instars feed on the foliage of *Tamarix*. When fully grown, the third instar larvae crawl or drop to the ground and pupate in the underlying leaf litter. Two and sometimes three generations can be produced in North America, prior to entrance of adults into reproductive diapause in response to shortened daylength (Bean *et al*. 2007). The adult stage overwinters in the leaf litter and becomes active around spring budbreak. Females oviposit 128 - 280 eggs in masses (3-8 eggs per mass) on the host foliage

(Lewis *et al*. 2003b, Milbrath *et al* 2007). In its native range, ***D. elongata*** herbivory can cause heavy to complete defoliation of *Tamarix* (DeLoach *et al*. 2003).

Host Impact and the Effects of Defoliation

For ***D. elongata*** to be a successful biological control agent, its defoliation must have a significant impact on *Tamarix* growth and survival. Leaf-chewing chrysomelid beetles have been used with great successes in weed biological control programs including the control *Hypericum perforatum* L. by *Chrysolina quadrigemina* (Suffrian) in California (Huffaker and Kennett 1959) and the control of *Sida acuta* Burman f. by *Calligrapha pantherina* Stål in Australia (Flanagan *et al*. 2000). Leaf-chewing insects have been important to successful biological control programs because they remove photosynthetic tissue, which reduces the ability of plants to maintain growth and vigor. The biological control of perennial woody plants such as *Tamarix* is thought to be more difficult than control of annual herbaceous plants because the large food reserves of woody plants often enable them to compensate for herbivory by defoliating insects. However, examples of successful biological control of woody plants exist. Black sage (*Cordia macrostachya* (Jacquin)) was successfully controlled by the leaf beetle *Metrogaleruca obscura* Degeer and the eurytomid gall wasp *Eurytoma attiva* Burks in Mauritius (Fowler *et al*. 2000) and Malaysia (Simmonds 1980). Additionally, the leguminous, woody tree *Sesbania punicea* (Cav.) Benth has been successfully controlled in South Africa by the combined effects of three introduced weevil species: *Trichapion lativentre* (Beguin-Billecocq), *Rhyssomatus marginatus* Fahraeus and *Neodiplogrammus quadrivittatus* (Oliver) (Hoffman and Moran 1998). Although three insect species

combined to control *Sesbania punicea*, Hoffman and Moran (1998) emphasize that it is the impact the agents have on the target weed rather than the quantity of insect species that ultimately holds the key to reductions in host plant densities.

The consequences of *D. elongata* herbivory on *Tamarix* growth and vigor are largely unknown. While *D. elongata* can completely defoliate *Tamarix* trees, plants can re-foliate within weeks of defoliation. To recover from defoliation, plants need adequate carbohydrate reserves to regenerate new leaf tissue (Chapin *et al*. 1990, Loescher *et al*. 1990). Stored carbohydrates in woody plants serve important roles in metabolism, growth, development of cold hardiness, defense and survival (Kozlowski 1992). Thus a reduction in carbohydrate reserves in *Tamarix* by *D. elongata* defoliation could lower plant growth and vigor.

The removal of photosynthetic tissue by defoliation has been documented to lower carbohydrate storage reserves in some plants. Reduction in carbohydrate reserves following artificial defoliation of eight salt-desert shrub species was the result of continued respiration, reduction in photosynthesis and the use of reserves in producing regrowth (Trlica and Cook 1971). Reserve carbohydrates were reduced while supporting new leaf growth following artificial defoliation of honey mesquite (*Prosopis glandulosa* Torr.) (Cralle and Bovey 1996). Contrary to these results, the transient effects on carbohydrate reserves and the rapid recovery of growth revealed the tolerance of healthy stands of hybrid poplar (*Populus* X *canadensis* cv Eugeneii) to outbreaks of the defoliating gypsy moth (*Lymantria dispar* L.) (Kosola *et al*. 2001).

Woody plants accumulate and store carbohydrate reserves during periods when supply exceeds demands for maintenance and growth (Oliveira and Priestly 1988, Kozlowski *et al*. 1991). Concentrations of nonstructural carbohydrates have been used to measure metabolic reserves in many plants including salt-desert shrubs (Trlica and Cook 1971), sugar maple (*Acer saccharum* Marsh) (Renaud and Mauffette 1991), poplar (*Populus* spp.) (Kosola *et al*. 2001), honey mesquite (*Prosopis glandulosa* Torr.) (Cralle and Bovey 1996), Chinese tallow (*Sapium sebiferum* L.) (Conway *et al*. 1999) and *Tamarix* (Sosebee 2004). Nonstructural carbohydrates (NCHOs) are accumulated and stored resources which can be remobilized to support biosynthesis for growth or other plant functions. Starch, sucrose and reducing sugars comprise the NCHOs, whereas cellulose, lignin and hemicellulose are primarily structural in nature and not available as reserves (Weinmann 1947, Loescher *et al*. 1990). Based on the plants' reliance on reserve carbohydrates following the stress of defoliation, we predict that defoliation by *Diorhabda elongata* will reduce *Tamarix* nonstructural carbohydrates. Additionally, we predict that insect defoliation will result in a reduction in spring above-ground regrowth which may ultimately lead to tree death.

The objectives of this study were to determine the impact of *Diorhabda elongata* defoliation on (1) the nonstructural carbohydrate concentrations of *Tamarix* root crowns in both manipulative field cage and non-manipulative natural experiments and (2) the spring above-ground regrowth in field cage experiments. These results will help determine the potential impact of *Diorhabda elongata* as an effective biological control agent of *Tamarix* species.

Materials and Methods

Insect Species

Insects used in the field cage experiment at Lake Thomas, Texas in 2004 and 2005 originated from beetles collected on *Tamarix* species 3km west of Sfakaki, Crete, Greece (latitude 35.38°N, longitude 24.6°E, elevation 7m) in April 2002. The insects released into the field near Lovelock, Nevada in 2001 originated from beetles collected from *Tamarix* species 7km west of Fukang, China (latitude 44.16°N, longitude 87.98°E, elevation 567m) in July 1999. Beetles collected from Crete were identified as *Diorhabda elongata elongata* (Brullé) and from Fukang as *Diorhabda elongata deserticola* Chen by I.K. Lopatin (Professor, Byelorussian University, Minsk, Belarus).

All imported beetles came through the USDA-ARS Exotic and Invasive Weed Research Unit quarantine facility at Albany, California where parasites, predators and other organisms were removed. Beetles (eggs and adults) were subsequently sent to the USDA-ARS Arthropod Containment Facility at Temple, Texas and maintained on cultivated *Tamarix* in laboratory and field cages where details regarding beetle biology (Lewis *et al*. 2003b) and host range (DeLoach *et al*. 2003, Lewis *et al*. 2003a, Milbrath and DeLoach 2006a,b) were examined. Voucher specimens of *D. elongata elongata* used in our field cage study and *D. elongata deserticola* from the Lovelock release site were deposited to the Texas A&M University Insect Collection, College Station, Texas (under lot nos. 663 and 665, respectively).

Manipulative Field Cage Experiment, Lake Thomas, Texas

The effect of *D. elongata elongata* defoliation on *Tamarix* nonstructural carbohydrates (NCHOs) and regrowth under field cage conditions was determined by caging beetles on individual trees in a *Tamarix* stand on the western end of Lake J. B. Thomas (latitude 32°36.5' N, longitude 101°14.1 W, elevation 675 meters) in Borden County, Texas (Fig. 4A). The field cage experiment was conducted during the 2004 growing season and replicated during the 2005 growing season. We used a randomized stratified block design with an individual *Tamarix* tree as the experimental unit. Only trees which could fit within a field cage without modifications were used. Cages were a square 3.3 m on the sides and 2 m in height. Each cage was covered with 20 X 20 mesh, Lumite fabric (Synthetic Industries, Gainesville, Georgia, USA). Cages facilitated the replication of beetle treatments by confining beetles and excluding insect predators which may inhibit *D. elongata elongata* population increases. Tree volume estimates were made from measures of canopy diameter and total height [volume = (π) x $(\text{diameter}/2)^2$ x (height)]. Trees were stratified into blocks based on estimated volume, and one tree from each block was randomly assigned one of the following three treatments: caged beetle treatment (20 mating pairs of *D. elongata elongata* per tree); cage controls (cages with no beetles added); and no-cage, no-beetle controls. The no-cage treatment controlled for experimental artifacts due to cage effects. The 2004 study included six replicates for each treatment. The 2005 study included trees not used in the 2004 study with 10 replicates for each treatment.

Prior to inoculation with beetles, core samples were taken from the tree root crowns (July 2004, July 2005) and analyzed for nonstructural carbohydrates as described below. Following defoliation, cages and beetles were removed from the trees and root crowns were re-sampled (September 2004, December 2005). To measure the regrowth potential of tree root crowns following defoliation, all above-ground tissue from the experimental trees was removed while the trees were dormant (December 2004, December 2005). Spring regrowth was quantified by removing, oven drying and weighing all new shoot and leaf biomass the following spring (May 2005, May 2006).

Non-manipulative Natural Experiment, Lovelock, Nevada

Heavy snowfall during 1982-1983 resulted in flooding along the Humboldt River in Nevada. As the waters receded from the terminal basin, *Tamarix* invaded the Humboldt sink. Natural Resource Conservation Service (NRCS) officials estimate more than 5000 hectares or 60% of the sink area is exclusively *Tamarix* canopy (Stevenson 1996). In the summer of 2001, approximately 1650 *D. elongata deserticola* adults were released into a monotypic stand of *Tamarix* in the lower Humboldt sink near Lovelock, Nevada (Fig. 4B). The release has resulted in temporally different and spatially segregated defoliation of *Tamarix* at the release site. By the end of summer 2002, the beetles had multiplied, dispersed and defoliated trees occupying an estimated 2 hectares surrounding the release origin. At the end of the 2003 and 2004 season, the beetles had defoliated trees occupying an estimated 200 and 1800 ha surrounding the release origin, respectively (Geraci 2006).

By the end of 2004, four areas within the terminal sink of the Humboldt River could be distinguished by the number of consecutive years the trees had experienced defoliation by *D. elongata deserticola*. In this study, we consider each spatially segregated area of defoliation a separate treatment: treatment 'three' includes trees from the 2 ha which had experienced beetle defoliation for 3 consecutive years; trees in treatments 'two' and 'one' had experienced 2 and 1 years of defoliation, respectively. Trees in treatment 'zero' had experienced little to no defoliation by the end of the 2004 season. Prior to spring bud break (March 2005), the root crowns of 30 trees were sampled from treatments two, one and zero, and 15 trees were sampled from treatment three using the method described below.

By the end of the 2005 season, the beetles had defoliated trees occupying an estimated 8100 ha (Geraci 2006). As a result, all previously sampled treatment trees had experienced one additional year of beetle defoliation including trees from treatment 'zero'. In early April 2006, prior to budbreak, the same trees sampled in 2005 were re-sampled and considered as the 2005 treatment plus one (*e.g.* trees from treatment 'three' in 2005 were considered as treatment 'four' in 2006). Few trees could be found in the lower Humboldt sink which had not been defoliated by *D. elongata deserticola* after the 2005 season. As a consequence, 15 trees from Pyramid Lake (another terminal basin approximately 75 km E-SE from the Humboldt sink sites) were sampled to serve as the 'zero' treatment in 2006 (Fig. 4B).

Tissue Collection and Enzymatic Analyses

Tissue for NCHO analysis was taken from the root crown of the trees. The root crown is defined as the tissue between the branching stems and the roots of a plant and is located at or just below the soil surface. The root crown has been noted as a carbohydrate storage organ in woody plants (Trlica and Cook 1971) including *Tamarix* (Sosebee 2004). Root crown tissue was removed using an 18 Volt cordless drill and a 20mm (¾") wood boring bit. The bark layer was removed from the point of sampling using the boring bit, the crown was bored 10mm deep, and wood shavings were collected in aluminum foil placed under the boring bit. Holes were plugged with bees wax to prevent infections from entering the tree wound. Tissue samples were stored on dry ice in the field. Upon return to the laboratory, the samples were heated to 100°C for 90 minutes to halt any innate enzymatic activity and then dried at 65°C for 72 hours to remove all moisture. After drying, each sample was ground separately using a Wiley mill fitted with a 40 mesh (0.5mm) screen. Samples were stored in air tight vials in the dark and at room temperature until carbohydrate analysis.

Root crown samples were analyzed for sucrose, glucose, fructose and starch concentrations using the enzymatic method (Smith *et al*. 1964, McBee *et al*. 1983, Kiniry 1993) with some modifications. A 0.25g sample of dry material was measured into a disposable 50ml centrifuge tube and extracted three times with 95% ethanol at 80°C for 30 minutes. The supernatants from each sample extraction were decanted and combined in a clean 50ml centrifuge tube. The resulting ethanol extractions contained the water soluble sugars sucrose, glucose, and fructose. Pellet residues were saved for

subsequent starch analysis. A colorimetric enzyme kit (#E0716260, R-Biopharm Inc., Marshall, MI, USA) and spectrophotometer (Spectronic 601, Milton-Roy, Ivyland, PA) were employed to determine sucrose, glucose and fructose concentrations from 200µl aliquots of the ethanol extractions. The residue pellets remaining after the aqueous extraction were resuspended in 15ml of distilled water and heated at 95°C for 30 minutes and then cooled on ice for 10 minutes. After cooling, 10ml of 100mM sodium acetate buffer (pH 4.5) and 20µl of amyloglucosidase (11,500 units/ml, A-3042, Sigma-Aldrich, St. Louis, MO, USA) were added to each sample, and the samples were digested for 24 hours at 55°C. Amyloglucosidase hydrolyzes starch to quantitative yields of glucose. From the digested starch solution, 100µl samples were analyzed for glucose, and starch concentrations were calculated from the glucose yields. Water soluble sugar (WSS) and starch concentrations were calculated as a percentage of sample dry weight, and tree NCHO concentration was reported as the sum of the WSS and starch concentrations.

Statistical Analyses

For the manipulative field cage experiment, proportional changes in NCHOs were calculated as ([post-treatment NCHOs] – [pre-treatment NCHOs]) / [pre-treatment NCHOs]. Data were transformed in cases where ANOVA assumptions for normality and equality of variance could not be verified from results generated by the Shapiro-Wilk test for normality and the Levene's test for equality of error variance. Manipulative field cage regrowth data were natural-log transformed, and natural experiment NCHO data were square root transformed. Two-way analyses of variance (ANOVA) were used to test for differences in proportional change in NCHOs and total

spring above-ground regrowth between treatments and between years in the manipulative field cage experiment (SPSS 11.5, SPSS Inc., Chicago, Illinois). Similarly, a two-way ANOVA was used to test for differences in NCHOs between years of defoliation and between sampling date in the natural experiment. When significant F-values were found, Fischer's protected least significant difference (LSD) test was utilized to separate significant ($P < 0.05$) mean differences. Mean regrowth and NCHO data were back-transformed for presentation. Additionally, the percent contributions of the WSS and starch concentrations to NCHO concentration were calculated in the natural experiment.

Results

Manipulative Field Cage Experiment

Experimental artifacts caused by cage effects were unlikely to have affected results as no significant differences in proportional change in NCHOs or spring regrowth between cage and no-cage controls (all $P \geq 0.05$) were found. Thus the no-cage controls were excluded and only the beetle treatment and cage controls were compared. No significant treatment-by-year ($F = 0.35$; df = 1, 31; $P = 0.56$) interaction effects were found for proportional changes in NCHOs, indicating the effects of cage control or beetle treatment on NCHOs did not vary with the year in which the experiment was conducted. Following defoliation, the cage control and beetle treatments did not differ significantly ($F = 0.38$; df = 1, 31; $P = 0.54$) in the mean proportional change in NCHOs (Fig. 5). NCHO concentrations increased 18.0 ± 9.2% and 12.1 ± 6.6% in the cage control and beetle treatment trees, respectively. Also, no significant treatment-by-year (F

= 1.35; df = 1, 31; $P = 0.25$) interaction effects on spring above-ground regrowth were found. However, the cage control and beetle treatments did differ significantly (F = 6.91; df = 1, 31; $P = 0.01$) in the mean spring above-ground regrowth (Fig. 6). Mean spring above-ground regrowth was 135.6 ± 17.3 grams in control trees and 89.7 ± 9.5 grams in trees which were exposed to beetles the previous season.

Non-manipulative Natural Experiment

Treatment (years of defoliation) by sampling date interaction (F = 9.20; df = 3, 222; $P < 0.001$) effects on NCHO concentrations were found in the natural experiment. Because of the interaction between the main effects, comparisons of NCHOs between treatments were analyzed separately for each year. Root crown NCHO concentrations differed significantly between treatments in 2005 (F = 34.58; df = 3, 102; $P < 0.001$) and in 2006 (F = 50.34; df = 4, 118; $P < 0.001$). In 2005, the mean NCHO concentration was significantly less ($P < 0.05$) in trees with at least one year of defoliation than in trees with no years of defoliation (Fig. 7). Mean percent NCHO concentration in trees which were not defoliated was 9.0 ± 0.8, compared to 3.2 ± 0.4%, 2.1 ± 0.4% and 2.3 ± 0.4% in trees defoliated for one, two and three years, respectively.

In 2006, the mean NCHO concentration was significantly less in trees which had experienced at least two years of defoliation than in trees which had experienced no more than one year of defoliation. Furthermore, NCHO concentration was significantly less in trees which had experienced one year of defoliation when compared to trees which had experienced no defoliation (Fig. 8).In 2006, mean percent NCHO concentration in trees which were not defoliated was 13.6 ± 0.9, compared to 7.6 ±

0.8%, 2.3 ± 0.4%, 1.5 ± 0.3% and 1.7 ± 0.4% in trees defoliated for one, two, three and four years, respectively. Starch contributed most to NCHO concentrations and was the component most reduced in trees which had experienced defoliation (Figs. 7 and 8). In 2006, starch contributed 84% and 78% to NCHOs in trees defoliated for zero and one year, respectively; whereas the starch contribution was 55%, 66% and 60% in trees defoliated for two, three and four years, respectively.

Discussion

Manipulative Field Cage Experiment

All experimental trees accumulated nonstructural carbohydrates in the root crowns between July and September 2004. This is in accord with the seasonal phenology of carbohydrates observed in many woody plants including *Acer saccharum* (Renaud and Mauffette 1991), *Prosopis glandulosa* (Fick and Sosebee 1983), *Prunus avium* L. (Clair-Maczulajtys *et al*. 1994), *Sapium sebiferum* (Conway *et al*. 1999) and *Tamarix* (Sosebee 2004). In general, carbohydrate reserves of storage organs decrease rapidly in early spring as the organs serve as carbohydrate sources to supply energy for budbreak, root growth and vegetative and reproductive development (Kozlowski 1992). Reserves usually reach a maximum in the fall when acquisition via photosynthesis exceeds allocation to growth, and they then begin to decline after leaf fall and throughout the dormant season as the plants must rely on these reserves for all metabolic activity, especially respiration (Loescher *et al*. 1990, Kozlowski 1992).

Though beetle treatment trees accumulated less NCHOs than did control treatment trees in the 2004 experiment (data not shown), analysis of the 2004 data did

not detect a significant difference in the mean proportional change in NCHOs between treatments. We speculate two reasons for this result: 1) the limited sample size and high variation in NCHO data obscured the trend and 2) the fact that there was little time lag between beetle defoliation and the post treatment sampling of the root crowns in 2004. Beetles were introduced on 16 July 2004. Very little defoliation was observed by 4 August. Trees were completely defoliated by 3 September, at which time we removed tissue from the root crown for post-treatment analysis. Richards (1993) suggested that herbivory has the strongest impact on plant growth and survival if a long delay occurs between loss of photosynthetic tissue and leaf regrowth. This suggestion may extend to the effects of herbivory on plant carbohydrate storage because the plant must draw on these stored reserves during the defoliated period. Since the beetle treatment trees were without photosynthetic tissue for less than one month before we re-sampled for NCHOs, few carbohydrate reserves probably were utilized by the plants.

For the 2005 experiment, we increased the sample size to 10 per treatment. We also increased the period of time between beetle defoliation and post treatment quantification of NCHOs in 2005 by sampling for NCHOs in December, 4 months following beetle treatment. However, beetle numbers did not increase significantly in 2005. Consequently, the trees were only lightly to moderately defoliated by the end of the season. The incomplete defoliation may have contributed to the non-significant treatment effect on NCHOs. It is also possible that a single late season defoliation event does not have an immediate effect on root crown NCHOs due to compensatory effects such as carbohydrate re-allocation from the root system.

Although root crown NCHOs were not significantly affected by the single late season defoliation event, the above-ground regrowth measured the following spring was significantly different between beetle treatment and control trees. Over the combined experiments, beetle treatment trees manufactured approximately 35% less above-ground regrowth following one defoliation event the previous fall than did cage control trees. Reichenbacker *et al*. (1996) also reported significant reductions in above-ground growth parameters following manual defoliation of hybrid *Populus*, but nonstructural carbohydrates were only mildly affected when defoliation levels were between 25 and 75%. These results suggest that above-ground growth may be a more sensitive indicator of mild defoliation stress. However, barring any further defoliation stress, it is possible beetle treatment trees are able to recover from the reduction in spring regrowth. Due to the limitations in the design of our study, we are unable to state if and when the beetle defoliated trees made a recovery in regrowth.

Non-manipulative Natural Experiment

Unlike our manipulative experiment, the non-manipulative, natural experiment allowed us to sample trees which had been defoliated for extended periods of time and under natural conditions. Extended defoliation by *D. elongata deserticola* at Lovelock, Nevada significantly reduced NCHOs in *Tamarix* root crown tissue. Results from the 2005 sampling indicate that mean NCHO concentration was more than 9% of total dry weight in trees which were not defoliated compared to less than 4% in trees which were defoliated for one or more years. In 2006, NCHO concentration was more than 13% in trees which were not defoliated, 7% in trees which were defoliated for one year and less

than 3% in trees defoliated two or more consecutive years. The significantly higher NCHO concentration in the non-defoliated trees in 2006 compared to the non-defoliated trees in 2005 raises questions regarding the use of trees from Pyramid Lake in the comparison. Though the Pyramid Lake trees were not significantly different in size, we cannot say how other variables at this site which might impact carbohydrate reserves (such as water and soil nutrient availability) compare to the sites in the Humboldt sink. Regardless, the exclusion of the Pyramid Lake trees does not negate the fact that NCHOs accounted for less than 3% of total dry weight in trees defoliated 2 or more years.

Of the individual carbohydrates quantified, starch was the predominant component to overall NCHO concentration, and it was the carbohydrate most reduced by defoliation. Starch levels were also significantly reduced in roots of *Acer saccharum* trees severely defoliated by insects (Wargo *et al*. 1972). The authors suggested that starch levels indicate changes in carbohydrate metabolism and perhaps the magnitude of physiological disturbance. Starch is considered the most important reserve carbohydrate in woody plants because it indicates when and where a carbohydrate surplus is present above current needs (Kozlowski 1992). In the present study, *Tamarix* trees defoliated for one to four years still had measurable quantities of starch and water soluble sugars. The presence of starch and water soluble sugars may not necessarily be an indication of continuing plant metabolism. Some carbohydrate stores may become inaccessible to woody plants with time because they are in dead cells and cannot be retrieved by the plant (Ziegler 1964). This suggests that NCHOs can be detected in dead trees.

The depletion of *Tamarix* root crown nonstructural carbohydrates due to extensive defoliation in the natural experiment suggests a reduction in the vitality of the trees. At Lovelock, an estimated 40% of trees in the 2 ha area which had experienced defoliation for 4 consecutive years failed to produce any foliage in 2006 and were considered dead (Dudley *et al*. 2006). If these trees did die, it may have been due to the exhaustion of carbohydrate reserves by repeated defoliation. Death of *Eucalyptus* species in Australian forests after repeated defoliation by phasmatids or psyllids (*Glacaspis* spp.) was purported to be due to the exhaustion of starch reserves to a level which did not support respiration and growth (Bamber and Humphres 1965). The European gypsy moth (*Lymantria dipar* L.), increases tree mortality as the intensity, duration and frequency of defoliation increases (Davidson *et al*. 1999). Additional studies are required to determine the minimum level of carbohydrate storage that results in *Tamarix* death.

Carbohydrate reserves are also important to reproduction, and the depletion of these reserves in plants by herbivores has been demonstrated to reduce reproduction (Chapin *et al*. 1990). Flower and fruit production were significantly reduced in the perennial herb *Aralia nudicaulis* the year following herbivory by moose (*Alces alces*) (Edwards 1985). Although direct measures of the effects of defoliation on reproductive fitness were not measured in these studies, we observed that *Tamarix* trees were never reproductively active at the time of defoliation. Thus defoliation and the reduction in carbohydrate reserves by *D. elongata* herbivory may reduce recruitment of seedlings and slow the spread of *Tamarix*.

Summary

Carbohydrate reserves are essential for plant survival (Loescher *et al*. 1990, Chapin *et al*. 1990). Maintenance respiration in living cells when photosynthesis is low or has stopped due to defoliation or deciduousness is dependent on adequate carbohydrate reserves, as is new spring leaf growth in all deciduous species (Loescher *et al*. 1990, Kozlowski 1992). The results from the manipulative field cages experiment at Lake Thomas, indicate that a single late season defoliation by *D. elongata elongata* did not significantly affect root crown NCHOs; however, above-ground regrowth was reduced the following spring. Additional studies are needed in Texas to determine if defoliation by *D. elongata elongata* will have significant impacts on NCHOs and subsequently reduce *Tamarix* survival. *D. elongata elongata* has successfully established at a release site near Big Spring, Texas. The widespread defoliation by the beetle at this site in 2005 and 2006 affords the opportunity for additional host impact studies.

The results from the natural experiment demonstrate that extended defoliation by *D. elongata deserticola* significantly reduces nonstructural carbohydrate reserves in *Tamarix* and that repeated defoliation in subsequent years prevents recovery of these reserves. The decline in tree NCHOs appears to be associated with a reduction in foliage growth and may affect tree survival and seedling recruitment. Forty percent of the trees which were defoliated for 4 consecutive years are believed to be dead. These results indicate that *D. elongata deserticola* has a significant host impact and suggests there is the potential for successful control of *Tamarix* at the Nevada site.

CHAPTER IV

CONCLUSIONS

The current status of *Diorhabda elongata* leaf beetles as biological control agents of *Tamarix* in the United States was evaluated using the four steps of progression defined by Harris (1991): establishment, biological success, host impact and control success. In Chapter II, we evaluated the establishment and biological success of *Diorhabda elongata elongata* (Brullé) at two release sites in west Texas. In Chapter III we evaluated the host impact of *D. elongata elongata* and *Diorhabda elongata deserticola* Chen in manipulative field cage and non-manipulative natural experiments in Texas and Nevada, respectively. The results from these studies shed light on the potential control success of *Tamarix* in Texas and the United States.

During the summers of 2003 and 2004, *D. elongata elongata* from Crete, Greece was released at two sites within the upper Colorado River watershed in Texas. Using the definition of establishment as the recovery of an agent for at least two years following its open field release (Harris 1991), *D. elongata elongata* successfully established at Beals Creek. Following its release at Beals Creek in 2004, *D. elongata elongata* was recovered during every census between 2004 and 2006, and the population dispersed at an increasing rate from the site of release. The established *D. elongata elongata* population has been biologically successful as demonstrated by the increasing number of host trees attacked and defoliated. By August 2006, the area of tree defoliation at Beals Creek exceeded 2 ha and included approximately 400 trees.

However, following releases in 2003 and 2004, *D. elongata elongata* has failed to establish at Lake Thomas. Results from other experimental releases of *Diorhabda* species have also been extremely variable, ranging from failure of the beetles to establish viable populations to outbreak populations which have defoliated thousands of hectares of *Tamarix* (Kazmer *et al*. 2006). The reason(s) for the failure to establish at Lake Thomas and the successful establishment at Beals Creek can only be surmised. The fact that the first observed open field population increase of *D. elongata elongata* at Beals Creek occurred at two trees nearest the on-site nursery cage suggest the adults beetles were attracted to conspecifics in the nursery cage. Aggregative behavior in the *Diorhabda* species group is supported by the recent isolation of an aggregation pheromone from *D. elongata deserticola* (Cossé *et al*. 2005). An aggregative behavior in *Diorhabda* species may increase reproductive success. Hopper and Roush (1993) suggest that many biological control introductions fail because Allee effects (reduced recruitment at low densities) drives small, introduced populations extinct. Allee (1931) suggested that processes such as defense against predators or habitat amelioration decline as density declines so that per capita population growth rate may decrease as density decreases. Below some threshold density, growth rate would fall below zero and the population would go extinct.

Predation may have reduced the released populations of *D. elongata elongata* at Lake Thomas to a density below the critical density needed to overcome Allee effects. Predation by passerine birds and general arthropod predators is hypothesized to be a major cause of establishment failure or limited population growth of *Diorhabda* species

at some release sites (Kazmer *et al*. 2006). Red imported fire ants (*Solenopsis invicta* Buren) are believed to be responsible for the low survival of beetles at four sites where *D. elongata elongata* was released in the upper Colorado River watershed in 2006 (Knutson *et al*. 2006). Though *S. invicta* was not observed at Lake Thomas, other potential general arthropod predators were observed including adult Coccinellidae, Reduviidae and Araneae. A study has been initiated to identify the spider fauna inhabiting *Tamarix* in west Texas and determine which may have an impact on limiting the success of *D. elongata elongata* establishment (Eric Knutson, New Mexico State University, personal communication).

Increasing the number of beetles released or adapting the method of release might lead to increases in establishment success. Adult beetles from the Beals Creek population are being distributed to other sites within the upper Colorado River and Pecos River watersheds. Between May and July 2006, more than 4000 adult beetles were released at 15 sites in 10 counties in west Texas (Knutson *et al*. 2006). The purpose of the study is two-fold: to assist in distributing the beetles to watersheds infested with *Tamarix* throughout west Texas and to determine which method of release will optimize establishment of *Diorhabda* at release sites. The methods include sleeve caging beetles on branches, caging beetles on single trees within 3.3m by 3.3m by 2m field cages and open field releases onto *Tamarix* trees without cages. Caging beetles at the time of release may minimize dispersal and predation and allow the beetle population to reach the threshold density necessary to sustain population growth, at which time the cage can be removed. The development of a synthetic *Diorhabda* aggregation pheromone and

delivery system may improve establishment by retaining and aggregating beetles at new release site and thus overcoming Allee effects.

A logical approach is to introduce agents from regions of the native range that match the climate of the release area in the introduced range reasonable well. While the results from Beals Creek suggest *D. elongata elongata* is well adapted to the climate and habitat at that site, this beetle may not be the best-adapted or only candidate in the *Diorhabda* species group for the highly varied climates and habitats in the southwestern US. In a review of the taxonomy and biogeography of the *Diorhabda* species group, Tracy *et al*. (2004) note that *D. elongata elongata* is indigenous to Mediterranean and temperate forests of Italy to southern Bulgaria and central Turkey and may not be adapted to desert and grassland habitats in the southwestern United States. Using habitat suitability index (HSI) models which included sixty environmental layers, Tracy *et al*. (2004) suggest *Diorhabda elongata sublineata* Lucas and southern populations of *Diorhabda carinata* Faldermann may be better suited to climates of the southwestern U.S. deserts and grasslands, whereas *D. elongata elongata* is better suited to coastal regions of Texas and California.

Complementing the HSI modeling and hoping to take advantage of the full geographic range of *Diorhabda* species currently held in experimental colonies in the United States (*D. elongata deserticola* from Fukang, China 44.16°N and Turpan, China 42.86°N; *D. carinata* from Karshi, Uzbekistan 38.86°N; *D. elongata elongata* from Crete, Greece 35.19°N; and *D. elongata sublineata* from Sfax, Tunisia 34.66°N), investigators have begun regional testing of the different ecotypes inside secure field

cages at sites across the United States ranging from latitudes 30-48°N (Dalin *et al*. 2006). The study was initiated at nine sites during the fall 2006, including one site near Borger, Texas (35.65°N), with plans to increase to sixteen sites in 2007. The experiment will provide knowledge as to which *Diorhabda* species are likely to establish and be successful biological control agents of *Tamarix* over the widespread range of infestations in the United States, including Texas.

Perhaps as important as latitudinal and climatic suitability of each biological control agent are the genotypes of the target weed at each site. We do not know if the different genotypes within the *Diorhabda* species group will vary in their ability to attack different genotypes and species of *Tamarix*. Such interactions may influence beetle establishment and the level of control achievable. Determining the *Diorhabda* host range within *Tamarix* will be difficult because *Tamarix* taxa are difficult to distinguish (Crins 1989) and hybridization has further complicated determining which taxa are naturalized in the US (Gaskin and Schaal 2003). Limited molecular genotyping has been conducted from trees near the Lake Thomas and Beals Creek release sites by John Gaskin (Principal Investigator, USDA-ARS, Northern Plains Agricultural Research Laboratory, Sidney, MT) using chloroplast and nuclear sequence data. Sixteen trees 2 km from the Lake Thomas release site were identified as *Tamarix chinensis* Loureiro, *Tamarix ramosissima* Ledebour or their hybrids. Five trees at the Beals Creek release site were identified as either *T. chinensis*, *T. ramosissima*, the genetically indistinguishable *Tamarix gallica* L. and *Tamarix canariensis* Willdenow. or their hybrids. However, with the limited information and without empirical studies, we

cannot say whether or not host genotype had a role in the successful and failed establishment at Beals Creek and Lake Thomas, respectively. If inconsistencies in establishment success continue at future release sites, it will be necessary to more closely determine the *Tamarix* genotypes and if *Diorhabda* species virulence varies with *Tamarix* genotypes.

Once establishment and biological success are achieved, the biological control agent must exert sufficient herbivore pressure on the target weed to decrease its biomass or reproductive capacity. Though one defoliation event by *D. elongata elongata* in the manipulative field cage study did not affect root crown nonstructural carbohydrate (NCHO) content, spring above-ground regrowth was reduced. Results from the natural experiment indicate a significant reduction in *Tamarix* NCHO content following extensive and repeated defoliation by *D. elongata deserticola*. Trees which had been defoliated for two or more consecutive seasons had less than 3% NCHO content compared to 8% and 16% in trees which had been defoliated one or no seasons. Approximately 65% of these trees failed to produce any green foliage in 2006. If these trees are dead, it may be because repeated defoliation exhausted carbohydrate reserves to a level which does not support respiration and growth.

The established *D. elongata elongata* population at Beals Creek and the manipulative field cage study suggest that *D. elongata elongata* has the potential for establishment, biological success and host impact in west Texas. The established *D. elongata deserticola* population in Nevada has certainly been biologically successful as determined by the thousands of hectares of *Tamarix* trees defoliated since 2002 (Geraci

2006). The reduction in NCHO content and the concurrent lack of foliage regeneration from these trees indicate that *D. elongata deserticola* is significantly impacting its host.

Control success is the fourth and last measurable step in the progression of a biological control agent and needs to relate to the objectives set out upon initiation of the project (Harris 1991). *Tamarix* management projects are being conducted by many separate agencies over many different states. The objectives of each project are likely to be different depending on the severity of the infested site, different management tactics in use and the stakeholders involved. The objectives need not be solely in terms of changes in weed density but can reflect economic and environmental costs such as savings on herbicide applications or the amount of water salvaged.

Control success by a biological control agent can be defined as (1) complete, when no other control method is required or used; (2) substantial, where other methods are needed but the effort required is reduced; and (3) negligible, where despite damage inflicted, control of the weed is dependent on other control measures (Hoffman 1995). Though measures of establishment, biological success and host impact are evident, it is too early to predict to what degree of control success *Diorhabda* leaf beetles will achieve or if they will be successful in all ecoclimatic areas where *Tamarix* is damaging. Complete success by one biological control agent over the ecoclimatic range of a target weed is not common (McFadyen 1998). Complete control of *Tamarix* by *Diorhabda* species over portions of the infested area is a reasonable goal. However, in some fringe areas, substantial control of *Tamarix* by *Diorhabda* species may be achieved with

complete control resulting from the combined effects of other control measures including additional biological control agents and herbicide applications.

Biological control of *Tamarix* may require the introduction of several agents over several years (DeLoach 1996). Testing has been initiated at overseas laboratories on some twenty species of insects (DeLoach *et al*. 2000). Seven of these have been received in quarantine at the USDA-ARS Arthropod Containment Facility at Temple, Texas and testing has been completed on two species other than the *Diorhabda* species group: a mealybug, *Trabutina manipara* Hemprich and Ehrenberg, from Israel and a foliage-feeding weevil, *Coniatus tamarisci* F., from France (DeLoach 1996). Pending approval for release by APHIS, *T. manipara* and *C. tamarisci* may complement *Tamarix* biological control by *Diorhabda* species by further damaging the plant and/or by attacking in areas where *Diorhabda* species are less effective than desired.

Herbicide treatments provide rapid and consistent control of saltcedar and are well suited to large, monotypic infestations that have a significant negative impact, such as infestations in and around water reservoirs. The *Tamarix* management program for the upper Colorado River watershed of Texas includes large scale chemical applications using imazapyr and rotary wing aircraft (McGinty *et al*. 2006). Beginning at the Lake Thomas dam and extending to the top of the Lake Spence watershed, an estimated 1600 hectares of riparian habitat in the upper Colorado River watershed were sprayed during 2005 and 2006, including Beals Creek from Moss Lake to its confluence with the Colorado River; spraying will continue in 2007 and extend to the Lake Spence dam to treat an estimated 2400 ha of Tamarix (McGinty *et al*. 2006).

Classical biological control is included as part of the *Tamarix* management program for the upper Colorado River watershed for several reasons: (1) chemical control by aircraft is not 100% effective and may not be appropriate for small localized *Tamarix* populations or where *Tamarix* is mixed with desirable vegetation; (2) some landowners may not approve of an herbicide application on their property; (3) the beetles have the potential to persist and suppress re-invasion of herbicide treated areas, whereas herbicides lack residual control; and (4) biological control is less expensive relative to herbicide treatments, and offers a long-term, sustainable approach to managing *Tamarix*. Control success of *Tamarix* by *Diorhabda* leaf beetles in the upper Colorado River watershed may largely be dependent upon the insects' achievement of persistent suppression in and outside of herbicide treated areas.

The established population of *D. elongata elongata* at Beals Creek is less than 10 km upstream from Moss Lake where herbicide treatment has begun. If populations of the insect do not reach herbicide treated areas on their own accord within the next couple of years, attempts will likely be made to distribute populations into treated areas. Future research at these localities will likely address the compatibility of these two control measures. If *D. elongata elongata* populations are sustained on localized populations of *Tamarix* and can suppress the re-invasion in herbicide treated areas, control success by *D. elongata elongata* and management success of the weed may be achievable. Such a scenario in the upper Colorado River watershed could serve as a model for other management programs in the United States.

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APPENDIX

TABLES AND FIGURES

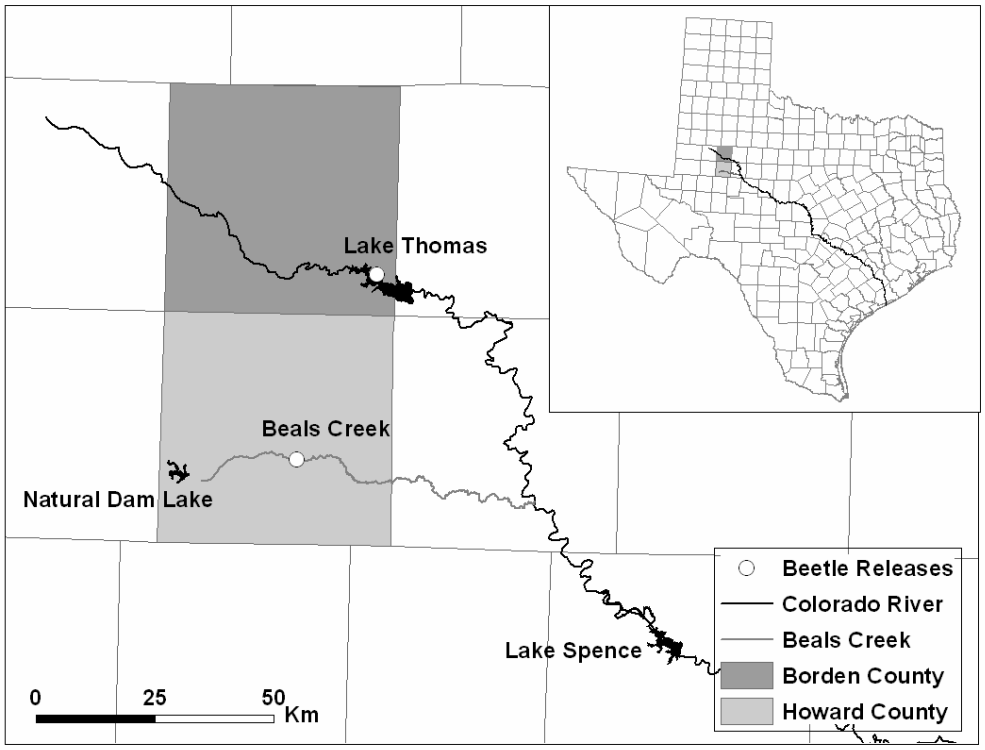

Fig. 1. Field release sites at Lake Thomas and Beals Creek within the upper Colorado River watershed of Texas.

Table 1. Date and number of *D. elongata elongata* released at the Lake Thomas and Beals Creek release sites in Texas 2003-2004.

Release Site	County	Release Dates	Insects Released
Lake Thomas	Borden	21 August 2003	1600 adults, 500 larvae
		16 July 2004	580 adults
		21 July 2004	400 adults
Beals Creek	Howard	22 April 2004	37 adults
		1 July 2004	141 adults
		6 July 2004	30 adults

Table 2. Abundance of *D. elongata elongata* insect stages observed at the Lake Thomas field release site in Texas 2004-2005.

Date	# Trees Surveyed	# Trees w/ *Diorhabda*	Egg Masses	Larvae	Adults
21 Apr 04	20	12	126	17	15
25 May 04	20	11	3	54	1
10 Jun 04	65	2	0	2	4
16 Jul 04	65	0	0	0	0
10 Aug 04	40	0	0	0	0
22 Oct 04	65	0	0	0	0
12 May 05	65	0	0	0	0
15 Jul 05	65	0	0	0	0

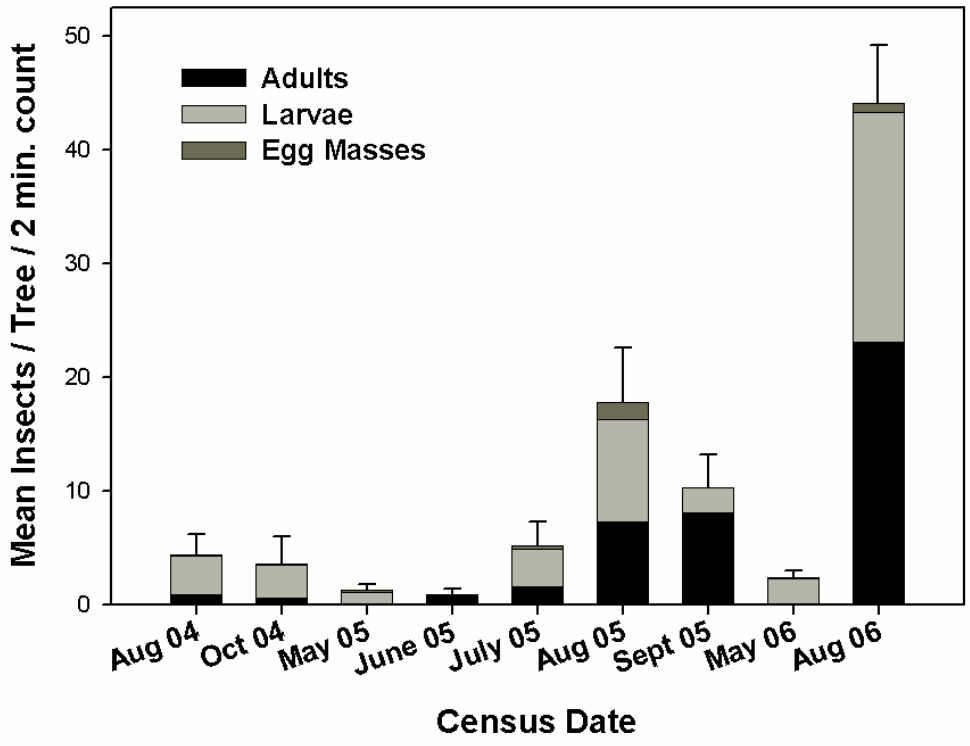

Fig. 2. Mean abundance of *D. elongata elongata* observed per tree searched per 2 minute observation at the Beals Creek field release site 2004-2006 (N = 47 trees per search). Standard error bars based upon total insect populations are shown.

Table 3. Mean and maximum dispersal distances and relative frequencies of trees occupied and defoliated by the *D. elongata elongata* population at the Beals Creek release site in Texas 2004-2006.

Survey Date	Mean Dispersal Distance (±SE) in Meters	Maximum Dispersal Distance (m)	Frequency of Trees	
			Occupied	≥ 90% Defoliated
3 Aug 2004	27 (0.7)	69	0.19	0.04
21 Oct 2004	42 (2.6)	69	0.19	0.08
11 May 2005	32 (1.9)	69	0.17	0
16 Jun 2005	24 (0.4)	28	0.06	0
7 Jul 2005	47 (1.2)	69	0.23	0.04
3 Aug 2005	55 (1.3)	221	0.62	0.21
9 Sep 2005	57 (2.0)	221	0.55	0.25
24 May 2006	81 (3.7)	177	0.4	0
17 Aug 2006	128 (1.5)	375	1.0	0.57

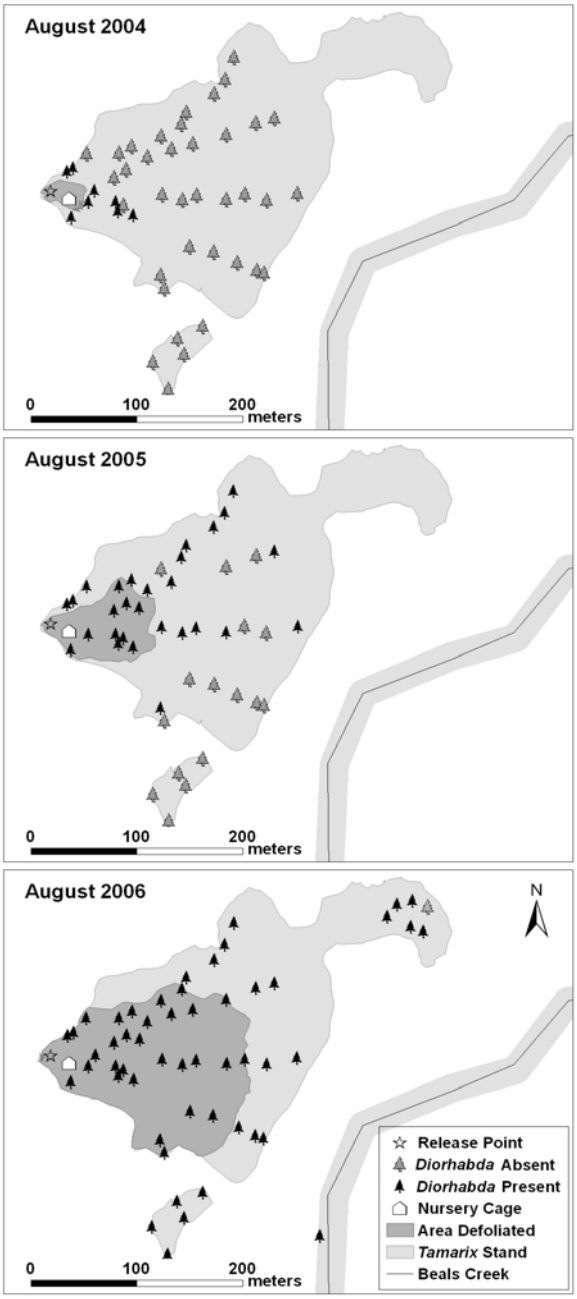

Fig. 3. *Tamarix* tree stand, location of trees surveyed along transects and progression of defoliated area in each August survey from 2004 to 2006 at the Beals Creek release site in Texas.

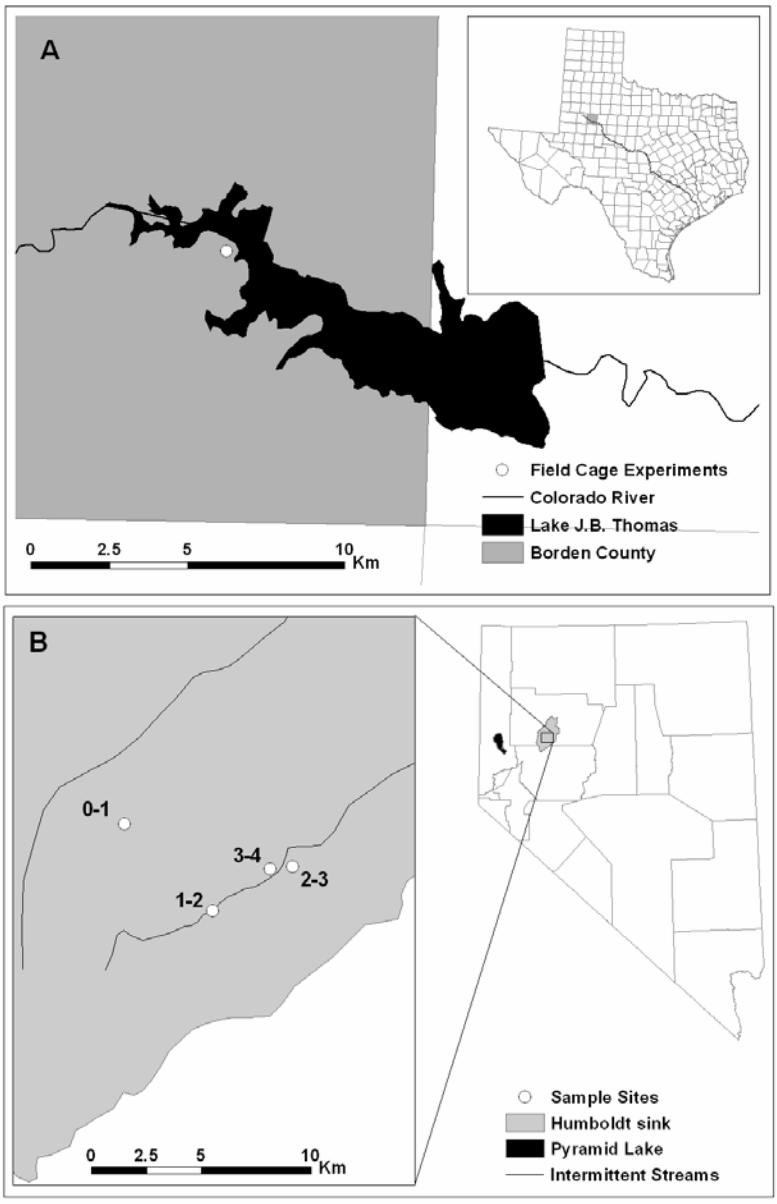

Fig. 4. Field cage experiment site near Lake Thomas in Texas (A) and natural experiment site in Nevada (B). Numbers next to Nevada sample sites indicate treatment: number of years of tree defoliation by *D. elongata deserticola* in 2005 and 2006. Trees at Pyramid Lake were sampled in 2006 to serve as 'zero' years of defoliation treatment.

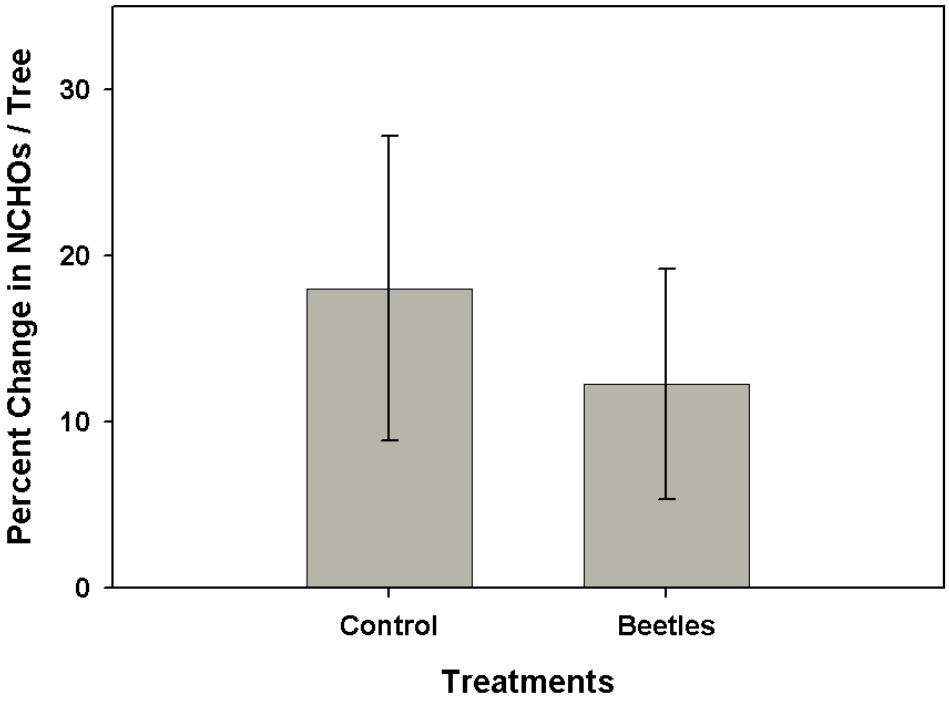

Fig. 5. Mean (± SE) proportional change in *Tamarix* root crown nonstructural carbohydrates (NCHOs) from the field cage experiment conducted at Lake Thomas, Texas in 2004 and 2005. Means are not significantly different ($P \geq 0.05$).

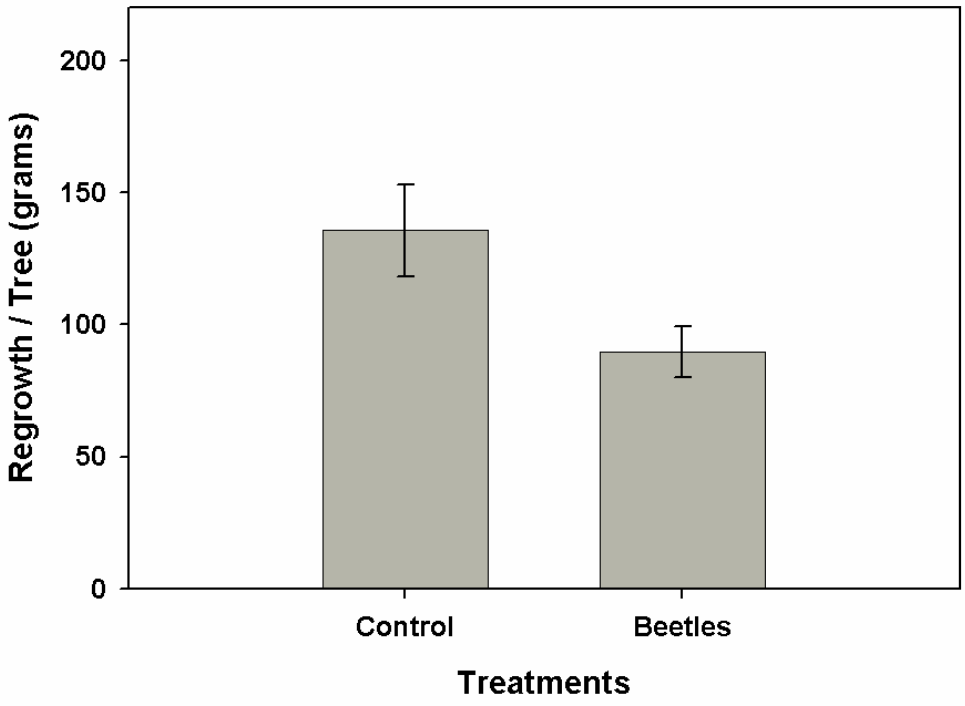

Fig. 6. Mean (± SE) spring above-ground regrowth of *Tamarix* trees from the field cage experiment conducted at Lake Thomas, Texas in 2004 and 2005. Means are significantly different ($P \geq 0.05$).

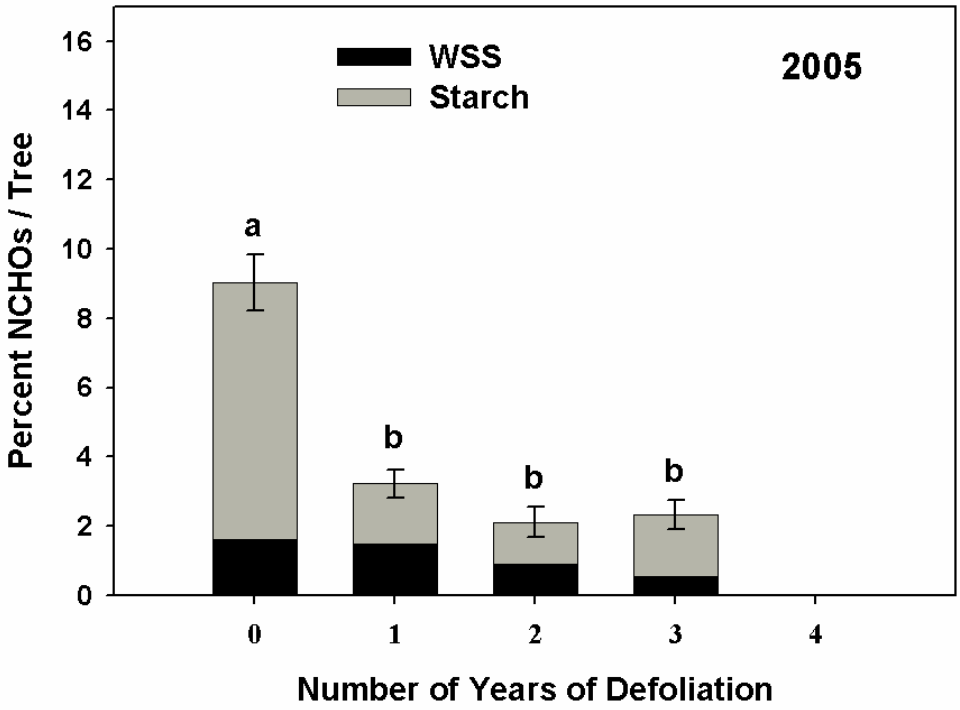

Fig. 7. Mean percent of water soluble sugars (WSS = fructose + glucose + sucrose), starch and nonstructural carbohydrates (NCHOs = WSS + starch) per *Tamarix* tree at the natural experiment site near Lovelock, Nevada in 2005. Standard error bars based upon NCHOs are shown. Mean NCHO with same letter above bars are not significantly different ($P \geq 0.05$).

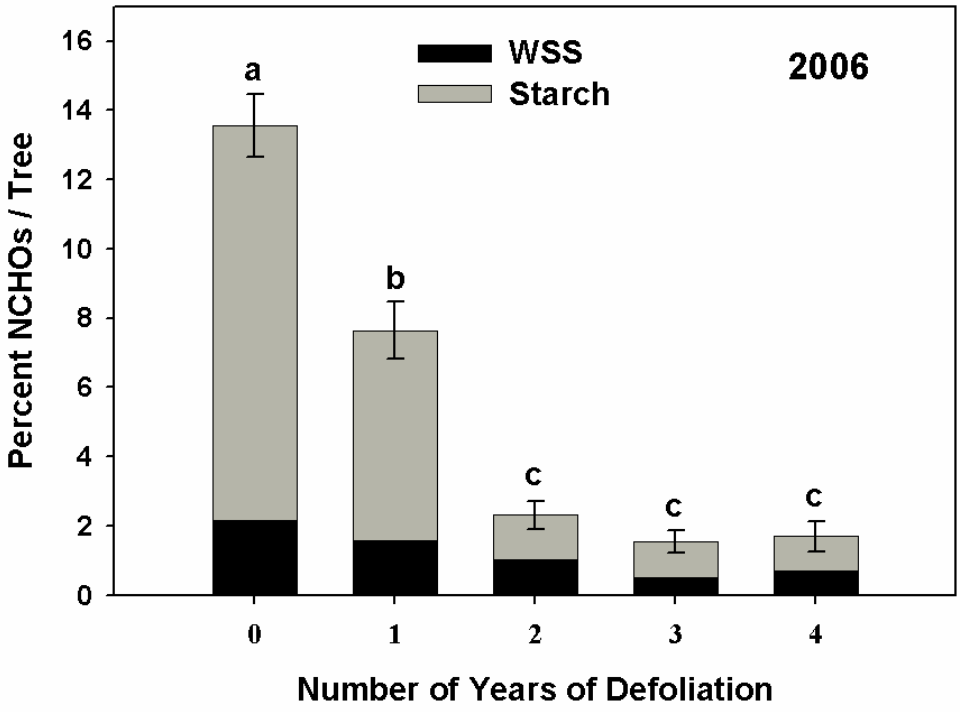

Fig. 8. Mean percent of water soluble sugars (WSS = fructose + glucose + sucrose), starch and nonstructural carbohydrates (NCHOs = WSS + starch) per *Tamarix* tree at the natural experiment site near Lovelock, Nevada in 2006. Standard error bars based upon NCHOs are shown. Mean NCHO with same letter above bars are not significantly different ($P \geq 0.05$).

VITA

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EDUCATION

Texas A&M University, College Station, Texas **2007**
Master of Science – Entomology
GIS Certificate through the Departments of Forest Science and Geography

Texas Tech University, Lubbock, Texas **1996**
Bachelor of Science – Molecular biology (Chemistry minor)
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EXPERIENCE

Texas A&M University **2003-2006**
Department of Entomology, College Station, Texas
Graduate Research Assistant

- Investigating the efficacy of the biological control of exotic and invasive saltcedar (*Tamarix* spp.) using the introduced leaf beetle *Diorhabda elongata*.

Graduate Teaching Assistant **Spring 2006**

- Organized and taught an upper division undergraduate laboratory dealing with pest and beneficial insects in the horticulture industry.

USDA/Agricultural Research Service **1998-2003**
Plant Stress & Water Conservation Laboratory, Lubbock, Texas
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- Investigated the genomics of desiccation (drought) tolerance within model plant species for future crop improvement.

HHMI Science Education Program **1992-1996**
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- Investigated the taxonomic relationships of New World bats within the family Phyllostomidae.

PUBLICATIONS

- Bogran, C.E., B.M. Drees, & J.L. Hudgeons. 2006. Gall-Making Insects and Mites. Texas Cooperative Extension Publication No. E-397. Agricultural Communications, The Texas A&M University System.

- Baker, R.J., V.A. Taddei, J.L. Hudgeons, & R.A. Van Den Bussche. 1994. Systematic relationships within Chiroderma based on cytochrome b sequence variation. Journal of Mammalogy. 75:321-327.